The nesting biology of *Ceratina* (Hymenoptera: Apidae) in the Niagara Region: New species, nest site selection and parasitism

by

Jess Vickruck, B.Sc.

Submitted in partial fulfillment
of the requirements for the degree of
Master of Science

Department of Biological Sciences, Brock University
St. Catharines, Ontario

Abstract

One of the most common bee genera in the Niagara Region, the genus *Ceratina* (Hymenoptera: Apidae) is composed of four species, *C. dupla, C. calcarata,* the very rare *C. strenua,* and a previously unknown species provisionally named *C.* near *dupla.* The primary goal of this thesis was to investigate how these closely related species coexist with one another in the Niagara bee community. The first necessary step was to describe and compare the nesting biologies and life histories of the three most common species, *C. dupla, C. calcarata* and the new *C.* near *dupla,* which was conducted in 2008 via nest collections and pan trapping. *Ceratina dupla* and *C. calcarata* were common, each comprising 49% of the population, while *C.* near *dupla* was rare, comprising only 2% of the population. *Ceratina dupla* and *C.* near *dupla* both nested more commonly in teasel (*Dipsacus* sp.) in the sun, occasionally in raspberry (*Rubus* sp.) in the shade, and never in shady sumac (*Rhus* sp.), while *C. calcarata* nested most commonly in raspberry and sumac (shaded) and occasionally in teasel (sunny). *Ceratina* near *dupla* differed from both *C. dupla* and *C. calcarata* in that it appeared to be partially bivoltine, with some females founding nests very early and then again very late in the season.

To examine the interactions and possible competition for nests that may be taking place between *C. dupla* and *C. calcarata,* a nest choice experiment was conducted in 2009. This experiment allowed both species to choose among twigs from all three substrates in the sun and in the shade. I then compared the results from 2008 (where bees chose from what was available), to where they nested when given all options (2009 experiment). Both *C. dupla* and *C. calcarata* had the same preferences for microhabitat

and nest substrate in 2009, that being raspberry and sumac twigs in the sun. As that microhabitat and nest substrate combination is extremely rare in nature, both species must make a choice. In nature *Ceratina dupla* nests more often in the preferred microhabitat (sun), while *C. calcarata* nests in the preferred substrate (raspberry). Nesting in the shade also leads to smaller clutch sizes, higher parasitism and lower numbers of live brood in *C. calcarata,* suggesting that *C. dupla* may be outcompeting *C. calcarata* for the sunny nesting sites.

The development and host preferences of *Ceratina* parasitoids were also examined. *Ceratina* species in Niagara were parasitized by no less than eight species of arthropod. Six of these were wasps from the superfamily Chalcidoidea (Hymenoptera), one was a wasp from the family Ichneumonidae (Hymenoptera) and one was a physogastric mite from the family Pyemotidae (Acari). Parasites shared a wide range of developmental strategies, from ichneumonid larvae that needed to consume multiple *Ceratina* immatures to complete development, to the species from the Eulophidae (*Baryscapus*) and Encyrtidae (*Coelopencyrtus*), in which multiple individuals completed development inside a single *Ceratina* host. Biological data on parasitoids is scarce in the scientific literature, and this Chapter documents these interactions for future research.

Acknowledgements

A huge thank you goes to my supervisor Dr. Miriam Richards. Over the last two years I have learned more than I thought humanly possible, not only about bees, but also how to think. Thank you for giving me direction when I was lost, but more importantly for giving me the tools to figure it out on my own.

Thank you as well to my committee members Dr. Gaynor Spencer and Dr. Fiona Hunter. Your questions and comments have been very helpful. Thanks also to Dr. Cory Sheffield for his assistance in bee identification, as well as Dr. John Huber for parasitoid identification and advice.

To all of my labmates and fellow graduate students in the Biology Department here at Brock, thank you for the memories...and the beer! Thanks especially to Sandra Rehan for 'bee'ing my crazy *Ceratina* partner in crime, as well as identifying specimens for me, and to Marianne Peso for showing me the ropes. To Chris Course and Rodrigo León Cordero, thank you for putting up with my sometimes strange and kooky behaviour.

I also owe a huge debt to my mom and dad for supporting their rather odd daughter, who would rather have caught insects than put on makeup. To mom for teaching me the value of reckless enthusiasm, and to my dad for teaching me how to think critically.

And lastly to Wes, for listening to me practice conference presentations even though you didn't really understand what I was talking about, telling me I could do it when I was sure that I couldn't, and celebrating the little victories with me along the way. I love you more every day.

Table of Contents

List of tables

List of figures

General introduction and thesis overview

Recent information suggests that factors such as climate change and habitat fragmentation are altering our local bee communities (Colla and Packer 2008, Grixti et al. 2009). Sadly, in most cases we know very little about the natural history and ecological interactions taking place with regard to the bee species being affected. Autecology, or the study of individual species and their interactions with the environment, is often overlooked due to the large amount of effort and small amount of recognition the research engenders (Murray et al. 2009). This information, however, forms the pivotal backbone for future research and assumptions that may be used in conservation analysis and efforts. This thesis examines the autecology of three species of *Ceratina,* or dwarf carpenter bees, in the Niagara Region.

Ceratina, (Hymenoptera: Apidae) are very common bees in the Niagara region, and the species that exist here have large ranges southward through to the eastern United States (Daly 1973). While the biology of *C. calcarata* has been the subject of several studies (Rau 1928, Johnson 1988, 1990, Rehan and Richards in press), there is little known about the other *Ceratina* species of eastern North America. The following thesis examines three species of *Ceratina* that are relatively abundant in the Niagara Region: *C. dupla, C. calcarata,* and a previously unknown species, *C.* near *dupla.* A fourth species, *C. strenua,* which is morphologically easy to identify, is rare in southern Ontario and none were collected over the two years of this study.

The first chapter of this thesis provides a detailed account of the life history and nesting biology of *C. dupla, C.* near *dupla,* as well as *C. calcarata* in the Niagara Region based on field sites studied in 2008. This information is also used to compare and

contrast the nesting biology of these three species, which superficially appear very similar in their biology and niches. This chapter provides the autecological background necessary to explore questions of competition examined in Chapter 2.

Ceratina dupla and *C. calcarata* are the dominant members of the bee community in Niagara. Their similar nesting biology and phenologies may lead to competition for important resources such as nesting sites. Are *C. dupla* and *C. calcarata* in competition for the same nest sites? Does this competition shape how nesting resources are used? Using the information gained in Chapter 1 on nesting site and substrate preferences of these two common species, Chapter 2 uses a nest choice experiment in combination with nest collections to explore the interactions of these two species and their nest site selection in the community.

The third chapter provides information on the parasitoid species affecting *Ceratina* in the Niagara region. Surprisingly little biological information is available on parasitoids, often due to their relative rarity. Descriptions of the developmental history as well as parasitoid interactions with their *Ceratina* hosts are documented, often for the first time. Prevalence and virulence in the nest, as well as species and nest substrate preferences are also examined.

A note on Ceratina nomenclature

Dr. Cory Sheffield recently informed me (on 19 January 2010) that the nomenclature of *Ceratina dupla* and *Ceratina* near *dupla* will soon be changed, based on his examination of a previously unavailable lectotype specimen (the holotype for *C. dupla* has been lost). The species referred to in previous literature and in this thesis as

Ceratina dupla, will soon be renamed *Ceratina mikmaqi*, while *Ceratina* near *dupla* will be known as *Ceratina dupla*. Since the name change is not yet official, I continue to use the currently valid names. The nomenclatural change does not affect the content of this thesis.

CHAPTER 1: The nesting biology of *Ceratina dupla* and a new cryptic species *C.* near *dupla,* with comparisons to *C. calcarata*

INTRODUCTION

Ceratina are the lone genus in the tribe *Ceratinini* (Apidae, Xylocopinae). All species share an affinity for nesting in wood. Unlike their larger, more robust subfamily-mates the *Xylocopa,* which are often found digging in hardwood, *Ceratina* nest in the exposed pith of twigs and stems. *Ceratina* are sparsely haired, often metallic bees and exhibit a wide range of body sizes from 2.2 - 12.5 mm (Michener 2007). The genus is comprised of 23 subgenera and is found on every continent (Michener 2007). While all *Ceratina* share the common trait of nesting in twigs and stems, social behaviour, phenology and morphology range widely.

All *Ceratina* species in the Niagara Region belong to the subgenus *Zadontomerus.* With a large distribution from Nova Scotia to British Columbia in the north, through the United States and Mexico to Venezuela, the subgenus *Zadontomerus* has a broad distribution (Daly 1973, Michener 2007, Sheffield et al. 2009). Bees from this subgenus have been described as weakly blue/green metallic, medium sized bees with typical body lengths of 5-7 mm (Daly 1973, Michener 2007). While the subgenus is composed of approximately 25 taxonomically described species, there has been relatively little biological research conducted on the group (J. Ascher, in Michener 2007).

Ceratina of the Niagara Region

Ceratina in the Niagara Region are comprised primarily of *C. dupla* and *C. calcarata,* which are among the 10 most common bee species collected in pan traps (Rutgers-Kelly 2003). Both species have large ranges encompassing most of eastern North America (Daly 1973, Michener 2007). *Ceratina dupla* and *C. calcarata* distributions overlap over almost all of their range. Due to difficulties in differentiating females, many previous studies have grouped these species together or based distributions entirely on males (Daly 1973, Tuell et al. 2008). The key of Rehan and Richards (2008) recently allowed for reliable distinction of the females for these two species. A third species, *C. strenua,* has also been collected in Niagara; however, they are extremely rare (Rutgers-Kelly 2003), and often years can pass without a single specimen being collected (Richards, pers. comm.) New evidence has shown that there is actually a fourth, cryptic species of *Ceratina* in the Niagara Region that is morphologically nearly identical to *C. dupla* (Rehan and Sheffield, in prep.) and which is relatively rare. Morphological traits have also been identified to allow for easier discrimination of males and females of all four species (Rehan and Sheffield in prep.)

Of the Niagara species, the only one that is well known is *Ceratina calcarata* (Rau 1928, Grothaus 1962, Kislow 1976, Johnson 1988, 1990, Rehan and Richards 2010). This ceratinine is univoltine, mass provisioning, and commonly nests in the exposed pith of raspberry (*Rubus* sp.), sumac (*Rhus* sp.), and cultivated rose (*Rosa* sp.) stems. Brood sex ratios are often male biased, and the innermost brood cell is usually female (Kislow 1976, Johnson 1988, Rehan and Richards 2010). While no natural

multifemale nests have been collected for this species, there is one account of coerced multifemale nests occurring in captivity (Chandler 1975).

The only study of *C. dupla* biology is an unpublished M.Sc. thesis focussing on a population in Georgia (Grothaus 1962). Unfortunately this study was very descriptive and mostly provided details of adult and larval morphology. Grothaus (1962) stated that *C. dupla* females provisioned nests with 11 or 12 brood cells, and he believed that occasionally females founded a second nest in the same season. Comstock (1911) also mentioned *C. dupla* in her nature field guide, where she reports that it has two generations per year in the northeastern United States.

Even this descriptive information is problematic. The largest issue is that until very recently, *C. dupla* and *C.* near *dupla* were grouped together as the same species. Interspecific differences would likely have gone unnoticed or would have been attributed to natural variation within populations. The ability to differentiate between *C. dupla* and *C.* near *dupla* now allows for the description of the biology of each species on its own.

Rationale and objectives

The bee community in Niagara is composed of approximately 120 documented bee species, including the four species of *Ceratina* (Richards, unpub. data). Superficially Niagara *Ceratina* appear to occupy similar niches in this community. They are closely related, share similar morphology, all nest in the exposed pith of twigs and stems, are polylectic (Rutgers-Kelly 2003) and have nearly identical ranges. In a bee community so diverse, it is likely that there is competition amongst some species, especially closely related ones such as the *Ceratina,* for important resources such as flowers and nesting sites (Potts et al. 2003, Potts et al. 2005). While this is probable, it is impossible to

investigate this competition without a basic understanding of the autecology of the species involved.

This study had two main objectives. The first was to describe the biology of *C. dupla* and the new *Ceratina* species (*C.* near *dupla*) in the Niagara Region. The second objective was to compare and contrast these biologies to each other and to *C. calcarata,* the other abundant *Ceratina* species in the area. Both of these objectives were addressed by collecting nests of all three species from the surrounding area, as well as by collecting flying bees in pan traps over the course of the nesting season in 2008. Investigating these three species in the same season allowed us to compare and contrast all three species with one another in an attempt to detect subtle differences between species without confounding environmentally based variation between breeding seasons.

METHODS

Field sites

Ceratina were collected at three sets of field sites located in St. Catharines, Ontario, Canada (43.1833N, 79.2333W) (Figure 1.1). Collection sites at the Brock University campus were in several abandoned old fields on the perimeter of campus, as well as along the margins of wooded areas. The Glenridge Quarry Naturalization Site (GQNS) was once a quarry that was restored as Carolinian natural habitat in 2003 and is composed primarily of hilly open fields. The northern edge of the GQNS borders the Bruce Trail where raspberry bushes and sumac stands can also be found. The field site at Glendale Avenue is an old field that has been abandoned for at least 6 years.

Figure 1.1. Map of collection sites in St. Catharines, Ontario. Brock University (BU), Glenridge Quarry Naturalization Site (GQNS) and Glendale Field (GF). Image courtesy of Google Maps.

Collection of foraging *Ceratina*

Foraging *Ceratina dupla, C.* near *dupla* and *C. calcarata* were collected in pan traps at five sites on the Brock University campus and at the GQNS (Figure 1.2) to help determine the flight phenology of each species. Insects, especially those that are interested in feeding on pollen or nectar, are attracted to the different coloured pans and then drown in the soapy water with which they are filled (Toler et al. 2005). My pan trapping protocol was based on the Bee Inventory Plot protocol (LeBuhn et al. 2003). Sites were chosen based on the criteria that they were a) near potential *Ceratina* nesting sites, and b) large enough for transects to be run through them. At each site two 50 m transects were established at a 90° angle to one another, forming a cross pattern. A stake was placed at the beginning and end of each transect to ensure the pans were placed consistently from week to week. Fifteen pans (plastic bowls SOLO PS6-0099) filled with soapy water were equally spaced along each transect, for a total of 30 pans (10 of each colour) per site. Each site was sampled once a week in random order from 14 April to 28 September 2008. Pans were set out at each site by 0900 h and brought in after 1500h. Insects were collected from the pans by straining them through a small sieve, after which they were rinsed with water. Specimens were then stored in 70% ethanol in 50 mL polypropylene centrifuge tubes labelled with the site and date. At a later time each sample was sorted, and the *Ceratina* specimens were separated, counted and preserved for additional analyses and measurements.

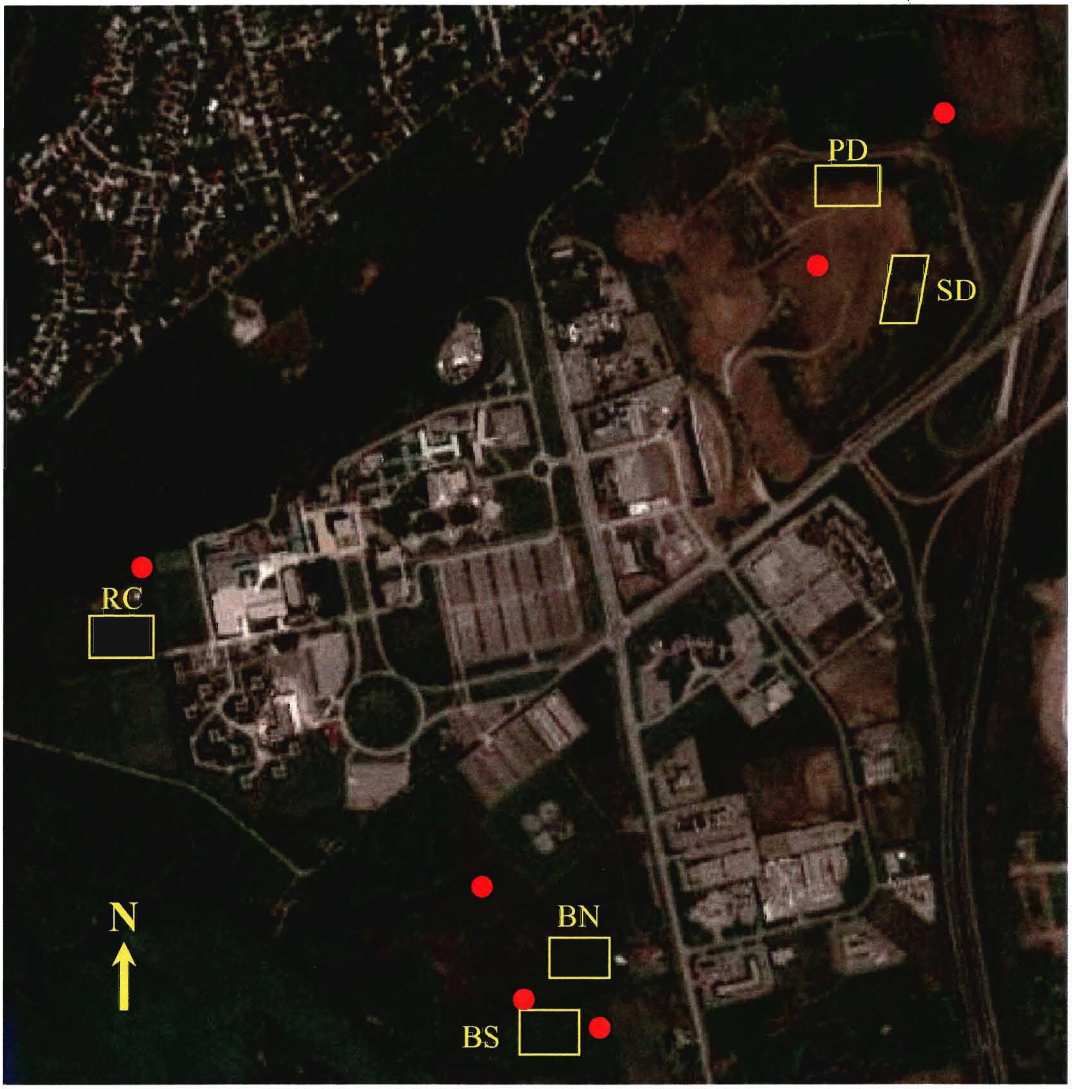


Figure 1.2. Aerial map of the five pan trap sites (yellow squares). Pond (PD) and St. Davids (SD) pan trap sites were located at the Glenridge Quarry Naturalization Site while the Ropes course (RC), Brock North (BN) and Brock South (BS) sites were located on the Brock University campus. Red dots mark the location of data loggers. Photo courtesy of Google Earth.

Nest collections

At least 15 *Ceratina* nests (10 *C. dupla*/ near *dupla* and 5 *C. calcarata*) were collected each week beginning 14 April and continuing to 16 September 2008, from the Brock University campus, the GQNS, and in the old field on Glendale Avenue in St. Catharines, Ontario (Figure 1.1). Nests were collected in early morning prior to the initiation of foraging to ensure that all occupants were inside. Possible nests were identified by a small hole (nest entrance) visible in the exposed pith at the end of small twigs and branches. After the entrance was covered with a small piece of masking tape, the twig itself was clipped with pruning shears 30-40 cm below the tape. Nests were brought back to the lab and put on ice for 15-20 minutes to cold anesthetise occupants. All nests were then carefully split open longitudinally to expose nest contents and leave cell septa intact. A schematic, labelled diagram of a typical nest cross-section can be seen in Figure 1.3. Nest contents including nest type, the number and sex of all adult occupants, the developmental stage and number of any immatures, and the presence of any parasites were recorded.

Based on their contents, nests were classified into one of six categories, modified from Daly (1966), as follows:

Hibernacula - Linear nests containing adults and varying levels of debris but without brood cells, pollen balls or larval faeces. Occasionally lines left from old cell partitions were visible.

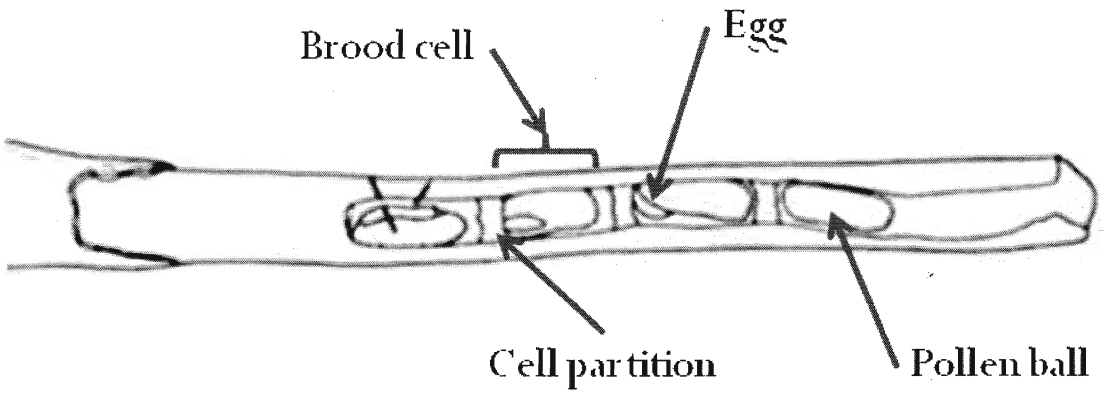


Figure 1.3. Schematic diagram of a typical *Ceratina* nest cross section. This would be classified as an active brood nest as the brood cell nearest the entrance is not complete.

New - Nests with bright walls (no old cell lines visible), no pollen balls, eggs or cell partitions.

Active brood - Nests containing at least one pollen ball and egg.

Full brood - Nests that met one of two criteria: a) brood cells filled the nest, leaving only enough room for the female to guard the entrance, or b) the brood cell nearest to the entrance contained at least a small larva, indicating that it had been at least 5 days since an egg had been laid.

Mature brood - Nests containing newly eclosed adults, immatures and usually a foundress (very worn female).

Rearing of immatures

All immatures (eggs, larvae and pupae) were reared to adulthood or death in the lab at room temperature (~21 °C). As each *Ceratina* egg is provisioned with all of the nutrients necessary to complete development, rearing immatures in the lab simply involved daily observations of each immature. Due to their fragility, larvae were left in the remaining (bottom) half of their nest after it was dissected, which was then covered with clear plastic tubing (ranging in diameter from ½-1 inch depending on the diameter of the twig) for protection. Once individuals had reached the pupal stage, they were transferred to 0.2 mL microcentrifuge tubes. Each immature was observed on a daily basis to assess developmental stage and day of emergence. Parasitized individuals were removed from the nest prior to the eclosion of parasites. Immatures were classified

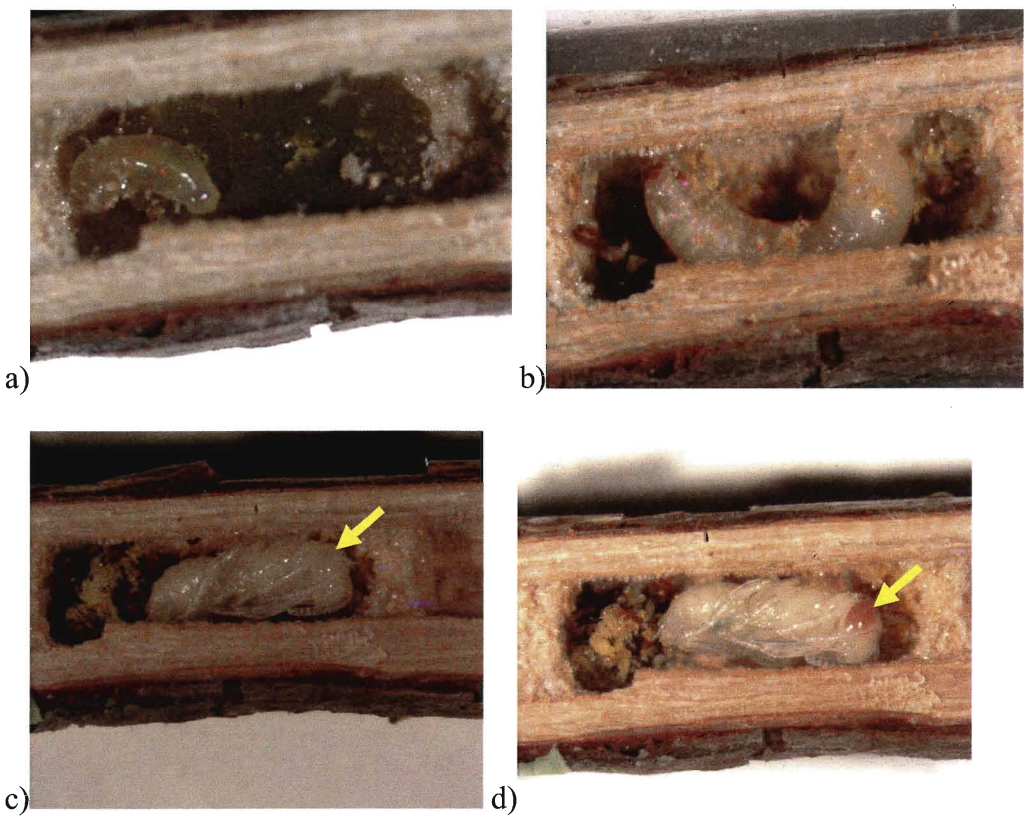


Figure 1.4. Stages in *Ceratina* development. A newly hatched larva (a) eats through its pollen ball until has consumed its entire mass and become a full grown larva (b). It then pupates to a white eyed pupa (c) after which the eyes change colour (arrows) (d).

into one of the 18 developmental stages originally described by Daly (1966a) for *Ceratina dallatoreana.* The first eight stages rank the larva in relation to the size of its pollen ball (Figure 1.4a, b), after which the larva passes through a pre-pupal stage. After pupation, the eyes of the pupa change from white to black (5 stages; Figure 1.4c, d), followed by darkening of the integument (outer skin; 4 stages). In the final stage the black-bodied pupa emerges as an adult with milky wings (a teneral adult).

Developmental rates were calculated by dividing the number of stages completed by the number of days taken to complete those stages. Individuals collected at the egg and prepupal stages were not included in developmental time analysis, as these stages are considerably longer than the others, and I could not be certain how far through the stage each newly collected individual had progressed when first collected.

Measurements

Adults were weighed on the day of collection and immatures were weighed on the day of emergence using a Mettler analytical balance (precision to 0.000 mg). Adult head width and wing length were also measured. Head width was measured across the widest portion of the face, including the compound eyes, at 40X magnification using a dissecting microscope fitted with an eyepiece micrometer (Fig. 1.5a). Wing length was measured using the subcostal vein length at the same magnification as head width (Fig 1.5b).

Wing wear was assessed as an approximation of cumulative flying time for bees collected as adults. Typically, wing wear is scored on a scale from 0-5, with scores of 0

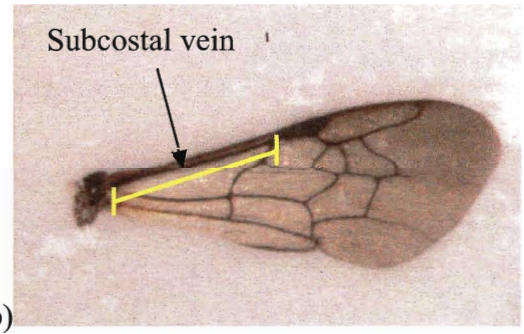
a)  b) 

Figure 1.5. a) Head width measurements were taken across the widest part of the face including the compound eyes. b) Wing length measurements were made by measuring the length of the subcostal vein.

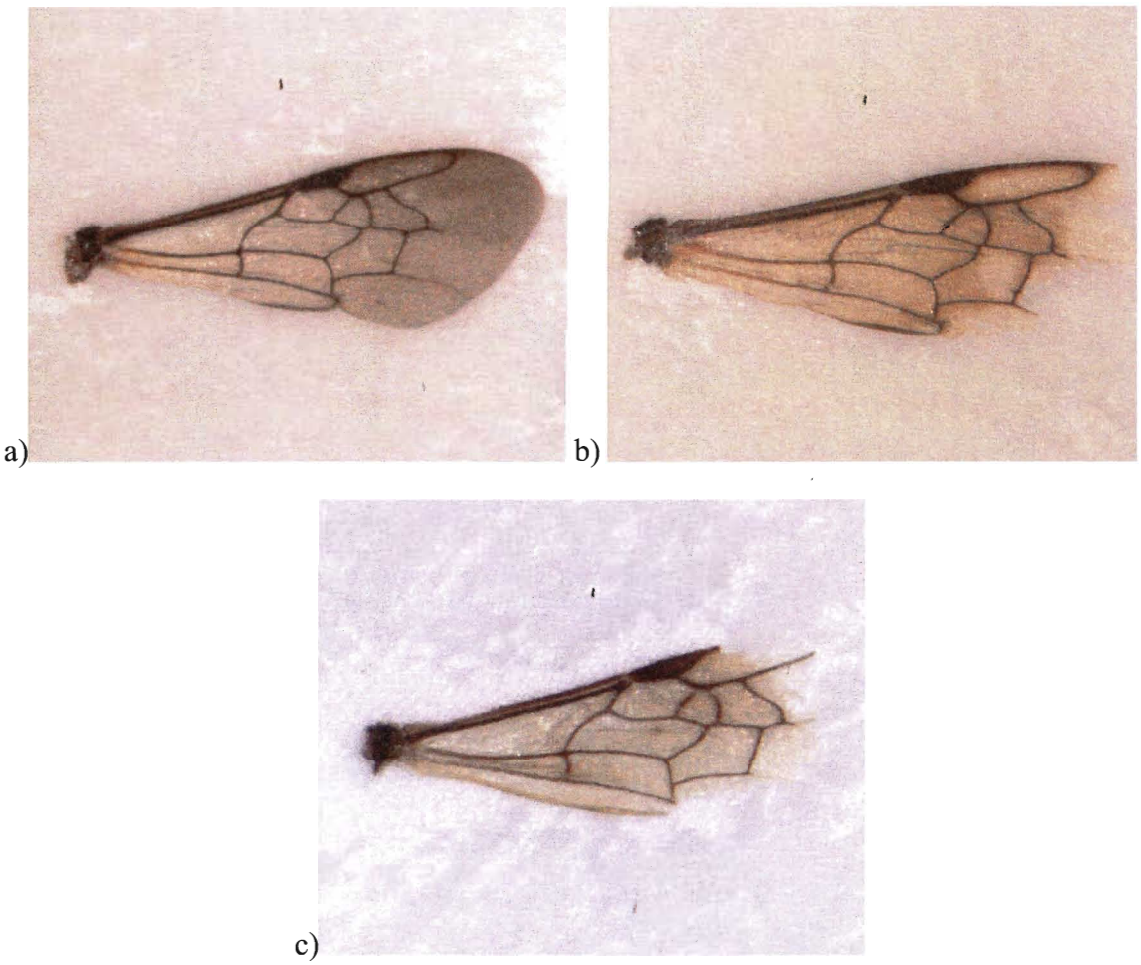


Figure. 1.6. Examples of wing wear for *Ceratina*. a) A wing with perfect wing margins and no nicks or tears received a score of 0. b) Wings with no visible margins remaining received a score of 5. In extreme cases wings were worn down to the point that wing veins were broken, or the cells themselves contained holes (c). These wings received a score of 6.

for individuals with completely new wings containing no nicks or tears along the wing margin (Figure 1.6a), and 5 for individuals having no visible wing margins left (Figure 1.6b). Due to the extreme wing wear of some individuals, an extra category of 6 was added. Wear on the wings of these bees had not only destroyed the entire wing margin, but had started to damage wing veins and cells (Fig 1.6c).

Data analysis

Data analysis was performed using SAS 9.1. All variables were normally distributed with the exception of development times, brood rearing success and wing wear. Parametric data were analyzed using parametric statistical functions with post-hoc Tukey tests where appropriate. Analyses of non-parametric data were based on ranks. Data are always presented as means ± standard deviation. Comparisons of pan trap distributions were made using Kolmogorov-Smirnov tests.

RESULTS

General description of *Ceratina* in Niagara

Pan traps were successful in collecting 336 foraging *Ceratina* females and 201 *Ceratina* males. Based on female pan trap collections, the total *Ceratina* community was 49% (164/336) *C. dupla,* 49% (165/336) *C. calcarata,* and 2% (7/336) *C.* near *dupla.* Based on males collected in pan traps the community was 79% (158/201) *C. dupla,* 16% (33/201) *C. calcarata,* and 10% (10/210) *C.* near *dupla.* No *C. strenua* were collected from pan traps during the 2008 season. Significantly more females than males were collected from pan traps ($G= 17.16$, d.f.=1, $P<0.0001$), however sex ratios from reared

brood did not differ from 1:1 (see section on sex allocation patterns) indicating that males of all species may be underrepresented in pan trap samples.

A total of 401 *Ceratina* nests were collected, comprising 178 *C. dupla* nests (67 hibernacula, 47 new nests, 21 active brood nests, 36 full brood nests, and 7 mature brood nests), 9 *C.* near *dupla* nests (1 active brood nest, 5 full brood nests, and 3 mature brood nests), and 207 *C. calcarata* nests (69 hibernacula, 69 new nests, 19 active brood nests, 42 full brood nests, and 8 mature brood nests). Nests always contained individuals from a single species of *Ceratina* with the exception of seven hibernacula which housed at least one *C. calcarata* along with either a *C. dupla* or *C.* near *dupla.* Nesting females of *C. calcarata* were commonly collected from raspberry (*Rubus strigosa*) (46% of nests) or teasel (*Dipsacus fullonum*) (36% of nests), and were somewhat common in sumac (*Rhys typhinia*) (18%). *Ceratina dupla* and *C.* near *dupla* females were both collected most often from teasel (80% of the time for *C. dupla*, 89% for *C.* near *dupla*), rarely in raspberry (20% and 11% respectively), and never from sumac. A more detailed analysis of site and substrate preferences will be presented in Chapter 2.

Some components of nest architecture were slightly different among species. Tunnel length did not differ (*C. dupla* 15.5±5.3 cm, *C.* near *dupla* 11.9±7.9 cm, *C. calcarata* 15.6±4.6; ANOVA $F_{(2,70)}=1.16$, n.s.), nor did tunnel diameter (*C. dupla* 3.5±0.4 mm, *C.* near *dupla* 3.6±0.3 mm, *C. calcarata* 3.6±0.4 mm; ANOVA $F_{(2,75)}=0.37$, n.s.). Brood cell length, however, was different, and *Ceratina* near *dupla* had significantly shorter brood cells (6.11±0.65 mm) than either *C. dupla* (7.41±1.4 mm) or *C. calcarata* (7.0± 1.2 mm) (ANOVA $F_{(2,87)}=5.37$, $P=0.006$). No hibernacula were

reused as nests by any species, and all females founded new nests by digging linear tunnels in twigs that had exposed pith.

The three species were distinct in their adult female body sizes ($F_{(2,337)}$=11.05, P<0.0001, Figure 1.7). *Ceratina calcarata* females were the largest (mean head width 1.96±0.16 mm), *C. dupla* females were intermediate (1.90±0.18 mm), and *C.* near *dupla* females were smallest (1.74±0.18 mm). Male head widths of *C. dupla* (1.67 ± 0.12 mm) and *C. calcarata* (1.70 ± 0.11 mm) did not differ (t=1.69, d.f.=1, P=0.09). No *C.* near *dupla* males were collected as adults in nests.

Flight phenology based on pan trap collections

Females

Pan traps were useful in revealing flight and wear patterns for males and females of all three species, especially *C. dupla* and *C. calcarata*. *Ceratina dupla* females began to emerge from hibernation during the week of 14 April 2008 (Figure 1.8a). The number of females caught in pan traps increased until the week of 2 June and decreased thereafter. High capture rates in June occurred as new and active nests were being collected (details given in next section), while the low capture rates in mid-July occurred when full brood nests were predominant. More females were caught again in late July and early August (Figure 1.8a). Nests collected at this time were mostly in the mature brood stage. Female wing wear increased over the course of the season as females spent more time flying (Spearman's rho=0.46, n=165, P<0.001). Seven females with wing wear scores of one were collected in pan traps in July and August and likely were newly emerged adults that were laid in 2008.

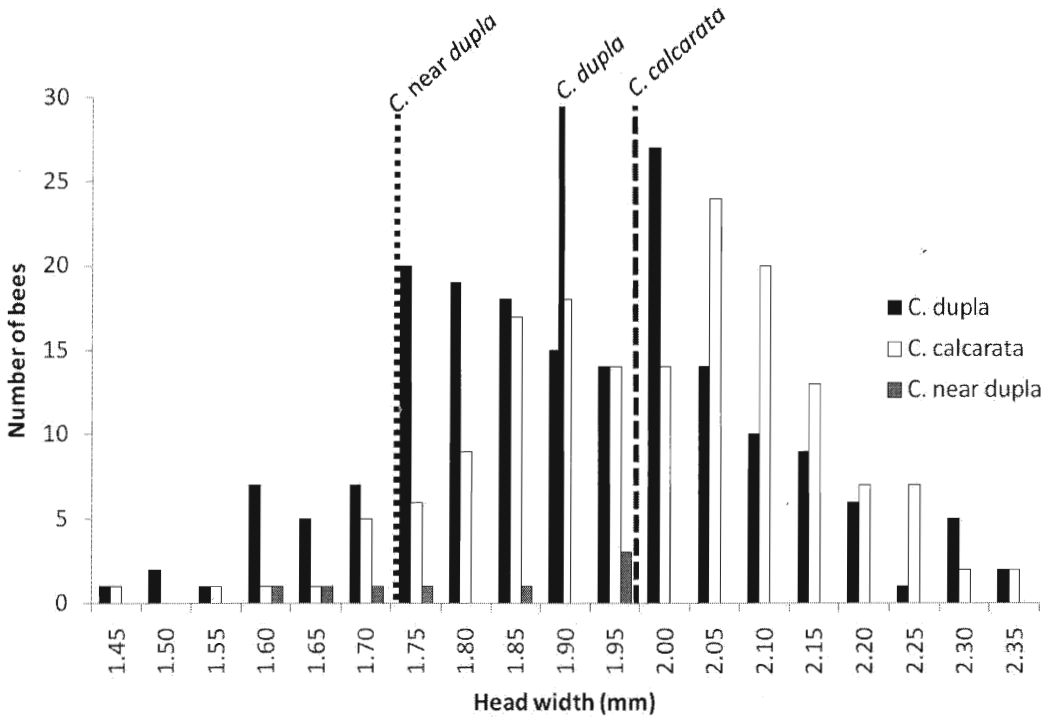
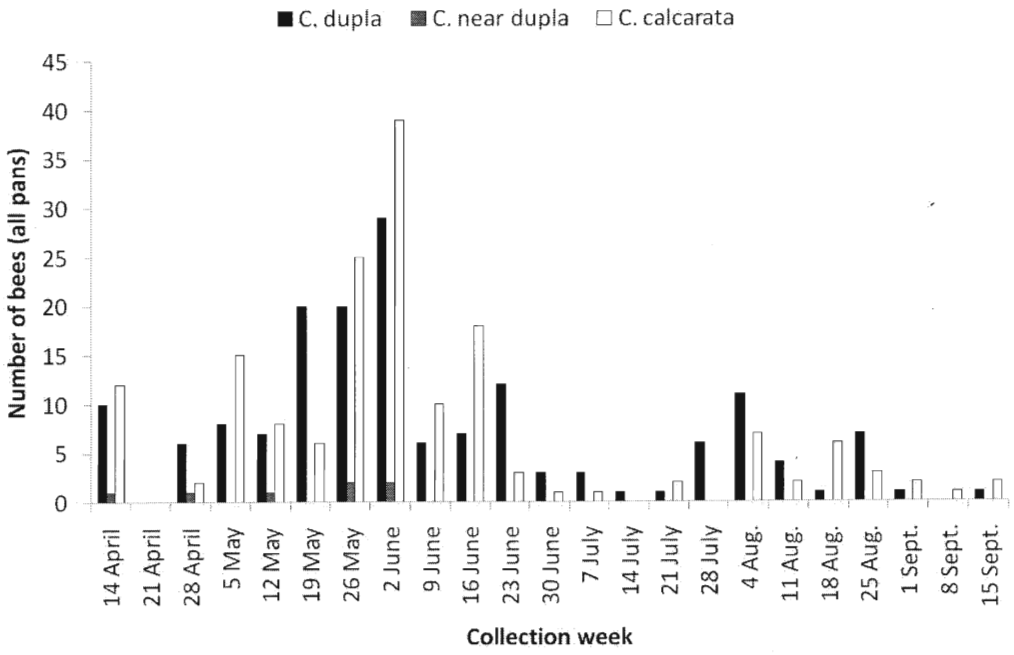


Figure 1.7. Body size distributions of female *Ceratina dupla* (black bars), *C. calcarata* (white bars) and *Ceratina* near *dupla* (grey bars) from nest collections in 2008. Lines indicate the mean head width for each species.

a) females



b) males

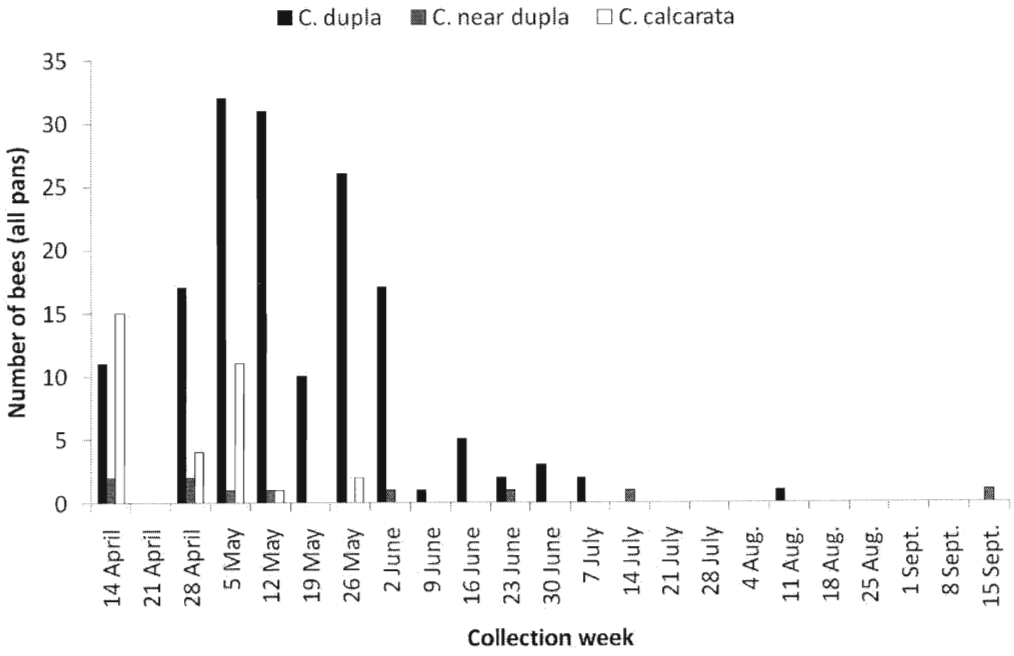


Figure 1.8. Flight phenology based on weekly pan trap collections of *C. dupla*, *C.* near *dupla* and *C. calcarata* (a) females and (b) males.

As only seven *Ceratina* near *dupla* females were caught in pan traps, inferences regarding flight phenology are somewhat difficult to make. *Ceratina* near *dupla* females had a significantly different distribution over the course of the summer when comparing cumulative number of specimens caught to either *C. dupla* (Kolmogorov-Smirnov D=0.70, KSa=2.35, P<0.0001) or *C. calcarata* (D=0.65, KSa=2.21, P<0.0001). No female *C.* near *dupla* were caught in pan traps after the week of 2 June (Figure 1.8a). The quiescent period after 2 June corresponded to full brood nest collections. Wing wear scores did not show the same changes in the season that were seen in *C. dupla* (Spearman's rho=-0.49, n=7, n.s.)

Ceratina calcarata females showed a similar pan trap abundance pattern to that of *C. dupla* (Figure 1.8a) (D=0.17, KSa=0.59, n.s.). Females emerged and were abundant in pan traps through the week of 2 June (Figure 1.8a). The numbers of foraging females then decreased, with very few females being caught in pan traps during the weeks of 23 June through 28 July (Figure 1.8a). Females were caught more frequently again in August. Females showed the same increasing pattern of wing wear over the course of the season as *C. dupla* (Spearman's rho=0.39, n=165, P<0.0001). Wing wear genereally increased as the season progressed, except for the appearence of unworn (newly emerged) females collected in pan traps in August and September.

Males

Ceratina dupla males with low wing wear scores emerged from hibernation during the week of 14 April, with captures peaking during the week of 5 May (Figure 1.8b). The number of *C. dupla* males declined steadily until the week of 7 July, after

which no male was collected for several weeks (Figure 1.8b). Wing wear increased significantly over the course of the season with the exception of one newly emerged male that was collected the week of 11 August (Spearman's rho=0.52, n=158, P<0.0001).

Ceratina near *dupla* males, while less abundant than *C. dupla* males, showed the same distribution pattern as *C. dupla* males (Kolmogorov-Smirnov, D=0.52, KSa=1.33, n.s.; Figure 1.8b). Unworn individuals emerged in mid-April, and two very worn males (wing wear scores of 6) were collected in June, with another male collected the week of 14 July. There was then a nine week gap when no males were collected until the last unworn, and likely newly emerged male was collected during the week of 15 September (Figure 1.8b).

Ceratina calcarata males had peak emergences during the week of 14 April. Wing wear was low but did increase over the season (Spearman's rho=0.37, n=33, P=0.03). The last *C. calcarata* male was caught during the week of 26 May, and unlike both *C. dupla* and *C.* near *dupla,* there were no males caught in August or September.

Nesting phenology

Ceratina dupla females emerged from their hibernacula and began to found new nests in early May (Figure 1.9a). Once this task was completed, females began to forage and return to their nests with pollen to make large provision masses. A single egg was laid on each provision mass. Active brood nests, which indicate the brood provisioning stage, were collected from 17 June through 14 July (Figure 1.9a). Full brood nests were

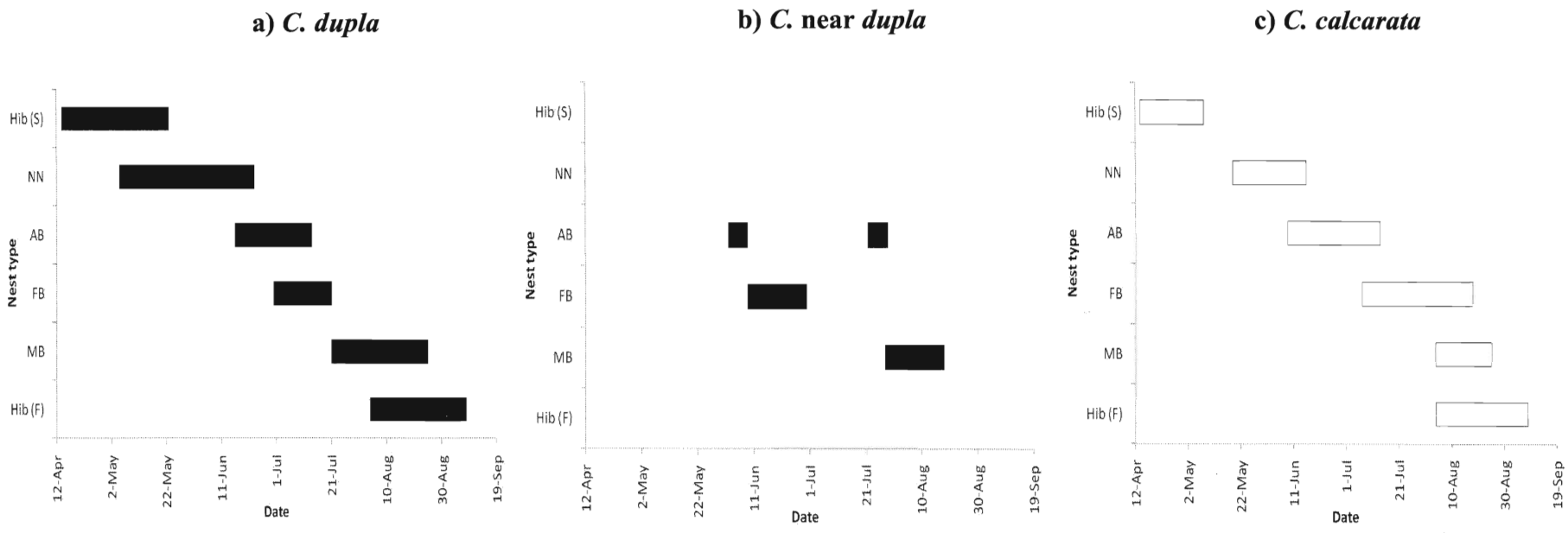


Figure 1.9. Nesting phenologies by nest type for (a) *Ceratina dupla* (b) *C.* near *dupla* and (c) *C. calcarata* from 2008 nest collections in the Niagara region. Black bars indicate the period during which nests of that type were collected. HIB (spring) - spring hibernacula, NN – new nests, AB – active brood, FB – full brood, MB – mature brood, HIB (fall) – fall hibernacula. Note that *Ceratina* near *dupla* full brood nests have been subdivided into two sections.

collected from 1 to 21 July, and mature brood nests were collected from 25 July to 27 August. The first hibernaculum was collected during the week of 4 August.

The first active brood nest of *C.* near *dupla* was the earliest collected of all species on 2 June, and the first full brood nest was collected only eight days later on 10 June. On 25 July and 1 August, two *C.* near *dupla* nests were collected that were very different from other nests of that time period (Figure 1.9b). One nest housed an egg and a larva that was the length of its pollen ball, and in the other nest was a dead larva and a fully grown larva. At a point in the summer when mature brood nests were being collected from *C. dupla* and *C. calcarata,* the eggs in these two nests would have had to be laid recently, so these were early, active brood nests. Based on the extreme wear of the foundresses (both with wing wears of six), these must have been females that had already raised a brood earlier in the season and had begun re-nesting to raise a second brood.

The nesting phenology of *C. calcarata* was very similar to that of *C. dupla* (Figure 1.9c). New nests were collected from 16 May to 18 June and active brood nests were collected beginning 10 June. *Ceratina calcarata* females had finished provisioning in mid-June, and full brood nests were collected from 8 to 21 July. This was followed by a period of collecting mature brood nests from 5 to 29 August. The first *C. calcarata* hibernaculum was collected on 8 August, and by September these were the only type of *C. calcarata* nest collected.

Brood productivity

Ceratina calcarata clutch sizes (7.56±4.04, n=42) were statistically smaller than those of both *C. dupla* (11.48±4.07, n=36), and the first brood of *C.* near *dupla*

(9.25±1.53, n=3) (ANOVA $F_{(2,77)}$=9.21, P<0.001). *Ceratina* near *dupla* also appeared to have a second brood with a mean clutch size of at least 2.0±0.0 (broods were not complete). Therefore the maximum lifetime reproductive success of a single foundress of *C.* near *dupla* would be approximately 11.25.

Brood parasitism occurred at different rates in the three *Ceratina* species (X^2=32.23, d.f.=2, P<0.0001). Least parasitized were *C. dupla* with 23% (101/437) of available cells in full brood nests parasitized. Common parasites included mites from the genus *Pyemotes* and chalcid wasps from the genera *Baryscapus* and *Axima.* The highest parasitism rates were found in *C.* near *dupla,* which was affected at a high rate of 60% (24/40 available cells). Interestingly, the only parasite found in *C.* near *dupla* nests was from the eulophid genus *Baryscapus. Ceratina calcarata* immatures had a moderate parasitism rate of 37% (109/295 available full brood cells). *Ceratina calcarata* was also parasitized by *Pyemotes, Baryscapus* and *Axima,* as well as *Eurytoma* sp. and a second species of *Baryscapus*. Detailed information on parasite development, abundance and host preferences is presented in Chapter 3.

As a result of parasitism and developmental failure, the average number of surviving brood per nest differed among the three *Ceratina* species, and the number of surviving brood was more variable than clutch size. *Ceratina dupla* had the most surviving brood per nest with a mean of 7.5±4.5 (n=36) bees. *Ceratina* near *dupla* had the lowest number of live brood with only 3.0±1.9 (n=3) individuals remaining, while *Ceratina calcarata* had a moderate number of live brood (4.0±3.2, n=42) (ANOVA $F_{(2,77)}$= 9.40, P<0.0002). Brood rearing success, or the number of surviving brood

divided by clutch size, was not different among species (*C. calcarata* 0.51±0.3, *C. dupla* 0.64±0.3, and *C.* near *dupla* 0.57±0.3, Kruskal Wallis H=3.40, d.f.=2, n.s.)

Brood developmental rates

Average developmental rates for *C. dupla, C* near *dupla* and *C. calcarata* respectively were as follows: 0.50±0.10 stages/day, 0.38±0.04 stages/day, and 0.54±0.19 stages/day. The time that immatures took to develop did not differ among species (Kruskal Wallis H=2.71, d.f.=1, n.s.)

Sex allocation patterns

The numerical brood sex ratios of both *C. dupla* and *C. calcarata* were slightly but non-significantly male biased (*C. dupla:* 125 females, 152 males, G=1.884, d.f.=1, n.s.; *C. calcarata:* 89 females, 99 males, G=0.745, d.f.=1, n.s). Only three *Ceratina* near *dupla* immatures (two males and one female) developed to the pupal stage, making sample sizes too small for this analysis.

In *C. dupla,* 21/27 sexable brood in innermost brood cells were female, a significant departure from an even sex ratio (G=8.83, d.f.=1, P=0.003) (Figure 1.10). This was also true for *C. calcarata,* where 26/34 individuals laid in brood cell one were female (G=10.03, d.f.=1, P=0.002). In addition, *C. dupla* female offspring laid in brood cell one were significantly smaller than their sisters in the rest of the nest (nested ANOVA $F_{(50,76)}$=2.40, P=0.003) (Figure 1.11a). The same pattern also was seen in

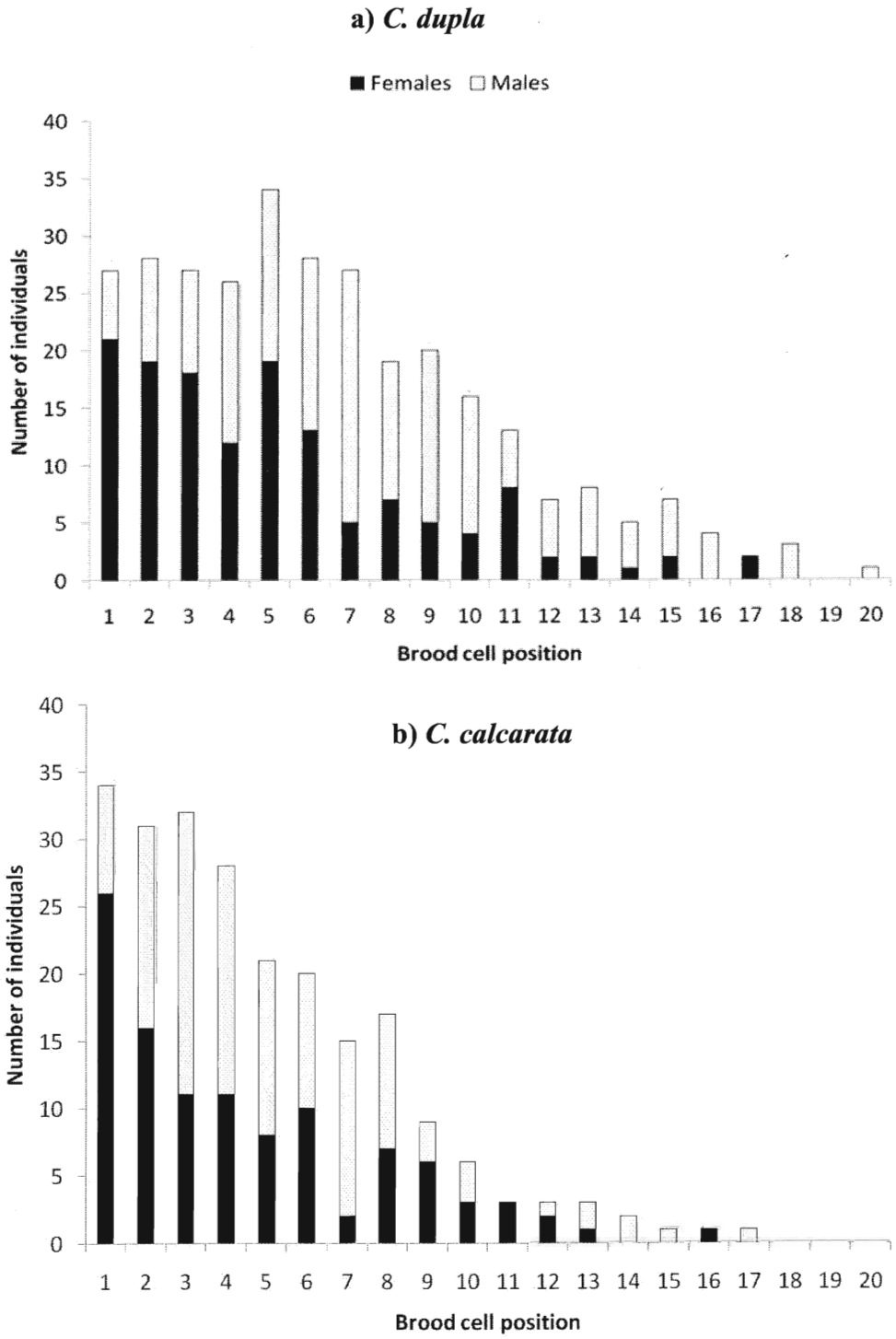
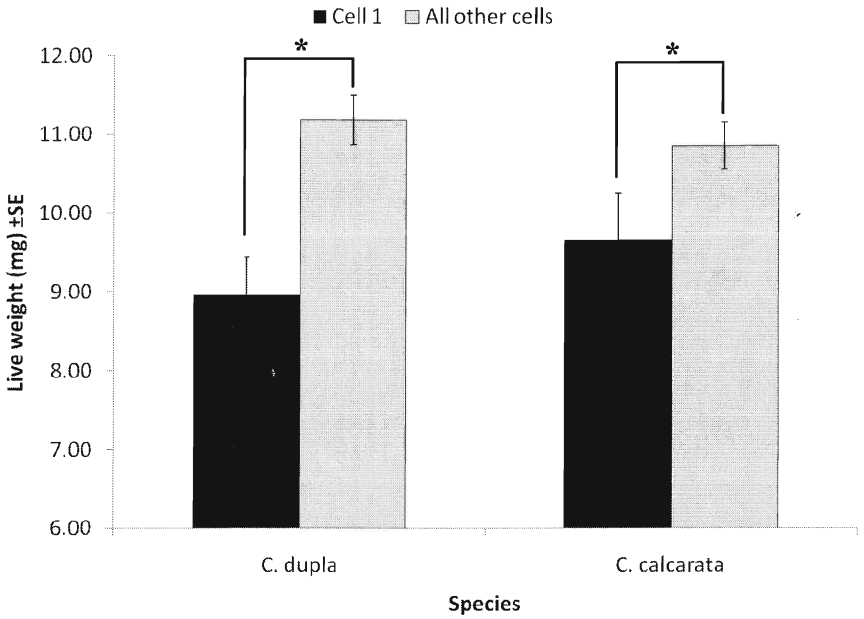


Figure 1.10. Sex ratio by brood cell position for a) *C. dupla* (n=277) and b) *C. calcarata* (n=185) with all sexable brood included. Unknown individuals are not displayed. Only three *C.* near *dupla* immatures emerged as adults and therefore could not be included in the analysis.

a) females



b) males

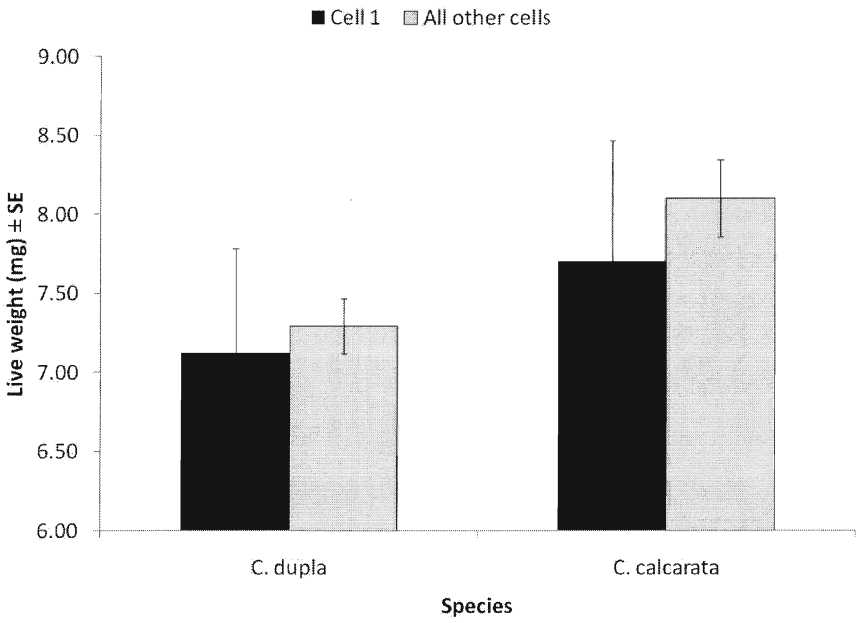


Figure 1.11. Live weight of a) female offspring ± SE and b) male offspring ± SE reared from *C. dupla* and *C. calcarata* nests. Females from brood cell one are smaller than their sisters, while this pattern is not seen in males. Only three individuals of *C.* near *dupla* emerged as adults and were not included in this analysis.

C. calcarata (nested ANOVA $F_{(52,33)}$=1.98, P=0.02)(Figure 1.11a). This size trend was not exhibited by male nestmates of *C. dupla* or *C. calcarata* (Figure 1.11b).

Maternal care and behaviour

Ceratina mothers of all three species exhibited very high survival rates during the nesting season. Of 68 *C. calcarata* nests collected in the active, full or mature brood stages, only three (4%) were orphaned. *Ceratina dupla* was very similar with only a 3% (2/64) orphaning rate. All nine of the *C.* near *dupla* active, full and mature brood nests collected contained a foundress.

Mothers of *C. dupla* and *C. calcarata* were occasionally found in the inner cells of the nest attending to brood. On July 8th a nest was opened with a female *C. dupla* residing in brood cell eight in a nest comprising 12 brood cells. If the split portion of the nest was almost closed she would resume her activities and could be observed for behavioural notes. One of the larvae in the inner cells had died, and after moving it to the bottom of the nest she proceeded to move all of the remaining offspring down one position further. She must have deconstructed the cell walls to gain entry to this portion of the nest. As she worked her way out she would push the rebuilt cell wall by backing up and pushing it with her abdomen. Females were found in the inner brood cells in 4 of 64 (6%) *C. dupla* nests and 3 of 68 (4%) *C. calcarata* nests. All nine *C.* near *dupla* nests contained adult females that were guarding when the nests were opened, however no females of this species were collected inspecting brood cells.

DISCUSSION

Life history and colony cycles of *Ceratina* in Niagara

Ceratina dupla is an abundant species in which both males and females overwinter as unmated, newly eclosed adults in twigs and stems. Mating occurs in mid-April, after which females dig new nests, most often in teasel stems. Once the foundress has dug a linear tunnel down the centre of the twig with her mandibles, she begins to forage for pollen and nectar provisions that she forms into large masses on each of which she lays an egg. The mother then forms a partition behind each provision mass using pith from the twig. She repeats this process, making multiple foraging trips, until she has finished provisioning. On average, a *C. dupla* female provisions 11.5 brood cells over the period from the end of June to the end of July. Once cell provisioning is complete, mothers cease foraging and sit at the nest entrance to guard their brood from predators and parasites. Foundresses also maintain contact with their offspring, entering the inner brood cells to incorporate larval faeces into the cell partitions and move immatures about in the nest. Despite this care, parasitism occurred at a rate of 23% in 2008. Brood begins to eclose at the beginning of August, with the innermost brood cell, which is usually female, eclosing first. After brood parasitism and death, *Ceratina dupla* females have a surviving brood of 7.5 offspring. Once brood has eclosed, foundresses and newly emerged offspring can be found outside the nest, possibly feeding or searching for hibernacula to overwinter in. The newly eclosed adults overwinter to begin the cycle again the following spring.

The mechanics of nest founding, cell provisioning and construction and nest founding in *Ceratina* near *dupla* are similar to *C. dupla*. *Ceratina* near *dupla* founds nests most often in teasel twigs and occasionally in raspberry twigs. Females had already begun to provision brood cells in early June and had completed their first brood by mid June. Two active brood nests collected late in the season (25 July and 1 August) are evidence for bivoltinism in this species. *Ceratina* near *dupla* nests also had very high parasitism rates which led to smaller numbers of surviving brood.

Ceratina calcarata nests were collected most commonly from raspberry and teasel twigs, and occasionally in staghorn sumac. Nest founding began in mid-May and full brood nests were collected in early July. The timing of nest founding and brood cell provisioning is very similar to that of *C. dupla*. *Ceratina calcarata* had a small clutch size (7.6) and moderate parasitism rates which led to low numbers of surviving brood. Further details of *C. calcarata* nesting behaviour are provided by Rehan and Richards (2010).

Interspecific differences

One of the most surprising contrasts among these three *Ceratina* species is the potential differences in voltinism (Table 1.1). Both *C. dupla* and *C. calcarata* are univoltine while the data suggests that *C.* near *dupla* may be bivoltine. Previously it was reported that some *C. dupla* females provisioned two nests per season (Comstock 1911, Grothaus 1962). As this is the first study to differentiate between *C. dupla* and *C.* near *dupla,* it is probable that the populations in those studies contained both species and may explain the results above. It has also been hypothesized that recently diverged species

Table 1.1. Comparison of important results comparing demographic and life history traits of *C. dupla, C.* near *dupla* and *C. calcarata* from this study as well from the literature.

Trait	*C. dupla*	*C.* near *dupla*	*C. calcarata*		
	This study	**This study**	**This study**	**Rehan & Richards (2010)**	**Kislow (1976)**
Location	Ontario	Ontario	Ontario	Ontario	Georgia
Voltinism	Univoltine	Bivoltine?	Univoltine	Univoltine	Univoltine
Most common nest substrate	*Dipsacus*	*Dipsacus*	*Rubus*	*Rubus*	*Rubus / Rhus*
Nesting begins	Early May	-	Mid - May	May	End of April
Brood emerges	Late July	Late July (2^{nd} brood?)	Late July	Late July	Late June
Clutch size	11.5	Brood 1: 9.2 Brood 2: 2.0	7.6	6.9	12.4
Brood parasitism (% of cells affected)	23%	60%	37%	15%	33%
Surviving brood per nest	7.5	3	4	4.1 (59% of 6.9)	6.9 (56% of 12.4)

may be more temporally isolated from one another than older, closely related species, and that temporal isolation is crucial to the speciation process (Rice 1987, Quinn et al. 2000, Friesen et al. 2007). This pattern is congruent with what we see between *Ceratina dupla* and *C.* near *dupla* where each species is very similar morphologically but differ most strongly in the timing of important events such as nest founding and provisioning.

Nest substrate was another characteristic that was found to be different among species. *Ceratina dupla* and *C.* near *dupla* were collected most often from nests in teasel and occasionally in raspberry. Grothaus (1962) mentioned that *C. dupla* nested in sumac, rose and bramble, however host species preferences were not reported. *Ceratina calcarata* differed in that it was collected most commonly in raspberry, but was fairly common in teasel and also nested occasionally in sumac. These results are fairly consistent with other work done on *C. calcarata.* Kislow (1976) reported collecting *C. calcarata* most often in raspberry and sumac, as did Rehan and Richards (2010; Table 1.1). Johnson (1988) also reported collecting *C. calcarata* from cultivated roses. No previous work has reported collecting *Ceratina* from teasel, and this is likely due to the fact that teasel is a relatively recent introduction to North America (Rector et al. 2006).

Differences in clutch size are also an important result of this study. Both *C. dupla* and *C.* near *dupla* have similar clutch sizes which are significantly larger than those of *C. calcarata.* Clutch size may be a result of nest location; *C. dupla* and *C.* near *dupla* were usually found nesting in teasel which is located in open fields, often in close proximity to wildflowers, whereas *C. calcarata* nesting in raspberry may have to fly further for each pollen trip. It has been shown that proximity to resources is positively correlated with the

number of brood cells bees can produce (Peterson and Roitberg 2006a, Peterson and Roitberg 2006b).

Ceratina dupla was also found to have significantly more surviving brood than either *C.* near *dupla* or *C. calcarata.* In Ontario, where the surviving brood for *C. calcarata* is ~4 (nearly half the number of surviving brood of *C. dupla*) this implies that nearly twice as many *C. dupla* offspring may found their own nests the following spring. It would be interesting to see if the *Ceratina* community composition changes over the next several years to reflect this result.

Intraspecific comparisons for *C. calcarata*

For *C. calcarata,* for which there is a larger body of work, comparisons can also be made between populations. Clutch size in *C. calcarata* appears to change with latitude (Table 1.1). In southern Ontario *C. calcarata* has a clutch size between 6.9-7.6, however the clutch size reported in Indiana was 10 (Grothaus 1962) and increased even more to 12.4 in Georgia (Kislow 1976; Table 1.1). Kislow also reported that nesting began for *C. calcarata* at the end of April, earlier than in southern Ontario. Warmer temperatures further south may lead to longer nesting seasons and larger clutch sizes, as also hypothesized by Rehan and Richards (2010).

Parasitism rates also appear to range widely. Even parasitism rates between this study and that of Rehan and Richards (in press), which both took place in the Niagara Region report parasitism rates of 37 and 15% respectively. Environmental conditions differed between seasons, with the summer of 2008 being particularly wet. *Ceratina australensis* also showed a relationship between environmental conditions and parasitism,

where hotter summers led to higher parasitism rates (S. Rehan, unp. data). Perhaps the wetter conditions in southern Ontario during the summer of 2008 allowed for parasites to thrive.

CONCLUSIONS

This study has described the phenology and nesting biology of *C. dupla* and *C.* near *dupla* along with *C. calcarata* in the same season. While all three species are morphologically quite similar and nest in the exposed pith of twigs and stems there are subtle differences among them. The most notable difference between *Ceratina dupla* and *C.* near *dupla* is the fact that phenologically they nest at different times; *C.* near *dupla* begins nesting earlier than *C. dupla* and may found a second brood later in the season. *C. calcarata* and *C. dupla* are very similar with regards to phenology, however they are collected primarily nesting in different substrates; *C. calcarata* from raspberry and *C. dupla* from teasel. These results suggest that *C. dupla, C. calcarata* and *C.* near *dupla* in the Niagara Region each occupy slightly different niches. Further studies into resource use, especially for nest sites, would be useful to understand how *Ceratina* species interact within the bee community in Niagara.

CHAPTER 2: Observational and experimental evidence for niche partitioning based on nest site selection in *Ceratina dupla* and *C. calcarata*

INTRODUCTION

Niches

The term niche is meant to define the specific environmental abiotic and biotic factors that allow for survival, growth and reproduction of a species. A word also used to describe a small indentation in the wall, it was first used by Grimmell (1917) in a biological sense to describe the environmental components that limited the range of the California thrasher. Elton (1927) defined the term niche independently of Grimmell, but his definition focused on the interactions of a species with others in its community, its "relation to food and enemies." The current use of the word niche brings both of these viewpoints together, combining environmental factors as well as the effects of competition, predation and parasitism. Gause's competitive exclusion principal further reshaped how biologists viewed niches (Gause 1934). His work with competing species of *Paramecium* demonstrated that two species sharing an identical niche cannot stably co-exist (Gause 1934). He thus concluded that in nature, two species cannot occupy identical niches without one driving the other to extinction. Therefore, according to Gause, if two species are found to co-exist they must occupy different niches.

Hutchinson (1957) further distinguished the term niche into fundamental niches and realized niches. A fundamental niche is described as a set of environmental factors necessary for a species to survive and reproduce (Hutchinson 1957). This is a useful definition, but it does not take into account that a species may share biotic and abiotic

factors with other species in its community. Hutchinson hypothesized that the fundamental niche may be altered by competition and interactions with other organisms that share overlapping preferences. The actual niche occupied by a species was termed its realized niche, which usually would be smaller than the fundamental niche for that organism (Hutchinson 1957).

Resource partitioning

One method by which sympatric species can reduce interspecific competition in niches is to somehow partition important resources such as food and nesting sites, so that each species uses the resource differently. A classic example of resource partitioning is Peter Grant's examination of competition between two species of Darwin's finches (Grant 1986). Beak size influences what types of seeds each finch species can consume, with the larger species with longer beaks (*G. magnirostris*) eating larger, harder seeds than the small species (*G. fuliginosa*), which eats smaller seeds (Grant 1986). By using different seeds as their primary food source, both species are able to reduce competition and thrive. A second example of resource partitioning can be seen in sympatric members of the ichneumonid genus *Megarhyssa* (Heatwole and Davis 1965). Females of this genus are obligate parasites of horntail larvae (Siricidae) that burrow in tree trunks. Females of *Megarhyssa atrata lineate, M. macrurus lunator* and *M. greenei greenei,* while otherwise similar, all have ovipositors of different lengths, and they divide resources by parasitizing horntail larvae at different depths in the tree trunk based on their ovipositor length (Heatwole and Davis 1965). In both these examples, competitors subdivide a particular resource based on subtle differences in morphology.

A second common type of resource partitioning is for sympatric species to subdivide the microhabitat found throughout their range. Gause (1932) showed that abiotic factors such as humidity and temperature were important in determining preferred habitat for different species of Orthoptera in the same community. Some species of spittlebug also divide resources in a similar manner (McEvoy 1986). Two species of spittlebug (*Philaenus spumarius* and *Lepyronia quadrangularis*) prefer the same plant structure for refuge, but they rest on leaf axils at different heights on the plant. *Lycaena helle* and *Proclossiana eunomia* are sympatric butterflies with declining populations in European countries (Turlure et al. 2009). While superficially both butterflies share similar habitat, *P. eunomia* prefers moister, darker, colder conditions (Turlure et al. 2009). This new information will now be taken into account when attempting to preserve the habitats of these species.

A third type of resource partitioning is temporal in nature. The halictid bees, *Evylaeus calceatus* and *E. albipes,* are very similar in terms of morphology and nest structure, but *E. calceatus* forages in morning and early afternoon, while *E. albipes* forages in early morning and then again later in the day (Plateaux-Quenu 1992). The ichneumonid wasps, *Reclinervellus tuberculatus* and *R. matsumotoi,* are closely related and share the same spider host (Matsumoto and Konishi 2007). Competition is reduced by *R. matsumotoi* completing development earlier than its sympatric competitor (Matsumoto and Konishi 2007). A similar story is revealed by two species of myrmecophilous butterfly, *Maculinea alcon* and *M. rebeli.* While these two butterflies have been shown to be genetically very similar (Bereczki et al. 2005), they have different

developmental rates as caterpillars, which leads to different flight and emergence times (Sielezniew and Stankiewicz 2007).

Evidence for competition within bee communities

Individuals in a community need access to food and nesting sites. In the case of bees this means that they are in need of plants from which they can obtain pollen and nectar, as well as sites appropriate to construct nests. Bees have been described as central place foragers, meaning that the location of their nest sites will determine what floral resources are within flight distance (Murray et al. 2009). Competition in bee communities has generally been studied in relation to partitioning of floral resources by bumblebees (*Bombus*) (Inouye 1978, Graham and Jones 1996, Goulson and Darvill 2004, Goulson et al. 2008). Several studies have supported the hypothesis that bumblebees reduce interspecific competition by partitioning floral resources according to tongue length (Inouye 1978, Johnson 1986, Graham and Jones 1996). Some studies found that bumble bees with longer tongues fed on flowers with longer corolla lengths than bees with shorter tongues (Johnson 1986, Graham and Jones 1996). This has led to the inference that the composition of the bumblebee community has been shaped by competitive interactions for floral resources.

It has recently been noted that nest sites may also play an important role in structuring bee communities (Potts et al. 2003, Potts et al. 2005). These communities are generally composed of several different guilds that have differing nesting requirements (Murray et al. 2009). Bees in the miner guild excavate tunnels in soft ground, and include the family Andrenidae, most Halictidae and Colletidae, and a few members of the

tribe Anthophorini (Apidae). The mason guild encompasses those groups which use pre-existing cavities for nests and includes most Megachilidae (Murray et al. 2009). The advanced eusocial nesters tend to use larger available nesting cavities and members of this guild are from the family Apidae (the genera *Apis, Bombus* and *Melipona* specifically). Last is the carpenter guild, whose members excavate nests by digging burrows into twigs or wood (Murray et al. 2009). Members of this guild comprise two genera from the family Apidae (*Xylocopa* and *Ceratina*) and one genus of Megachilidae (*Lithurgus*).

Factors affecting nest site selection in the Hymenoptera

A female's decision about where to lay her eggs has direct consequences for her future fitness. It is therefore one of the resources for which competition may be of primary importance. For this reason, an immense amount of work has been done investigating the factors surrounding nest site selection. The definition of a good nest site differs from organism to organism, and proximity to floral resources, camouflage from predators, nest microclimate, and nest substrate are just some of the factors affecting nest site selection in Hymenoptera.

Where a nest is located may significantly influence environmental conditions such as temperature and humidity that the nest, and therefore the individuals inside experience. As aculeate Hymenopteran larvae are largely immobile, they are restricted to the nest chosen and constructed by their mother until they emerge as adults. Brood in nests that get overly warm or which are in arid landscapes may be prone to desiccation or heat shock (Hranitz and Barthell 2003, Barthell et al. 2004, Hranitz et al. 2009), while brood

in nests located in cold, moist areas may have slower developmental rates and may also be more prone to mold. When given the option to construct nests in artificial environments set at different temperatures, the ant species *Myrmica punctiventris* chose the cooler temperatures over warmer, which may have buffered colonies from extreme heat (Banschbach et al. 1997). In contrast, the sweat bee *Halictus rubicundus* preferred sites that were more exposed to sun during the day, which in turn increased soil temperatures (Potts and Willmer 1997). These opposite reactions to sun may be attributed to the substrate in which these two species nest. *Halictus rubicundus* is a ground nester and the soil provides excellent insulation, whereas *Myrmica punctiventris* nests in twigs which are not good insulators. Moisture level was an important factor to the ground nesting bee *Dieunomia triangulifera*, which preferred moist soils as nest sites (Wuellner 1999).

The substrate from which nests are constructed is an important component of nest site selection. Many species have specific preferences for species of plant or composition of soil, while others seek out particular characteristics that make certain substrates more or less desirable. *Osmia cornuta* demonstrated a preference for specific artificial and natural substrates such as wood blocks and bamboo reeds, in which they produced significantly more female offspring (Bosch 1994). Two sympatric species of subtropical polistine each preferred to nest in different species from the *Acacia* family (Dejean et al. 2001). This was of particular interest due to the fact that *Acacia* trees are also occupied by arboreal ants, which may have provided protection for the wasps. While the nests of *Mischocyttarus collarellus* (Vespidae) were found in numerous different species of tree,

the common factor was that nest cavities were not vertical and that they were lacking in epiphytes (Smith 2004).

For many trap-nesting bees which use pre-existing holes, the size of the cavity can have an impact on variables such as clutch size, size of offspring, sex ratio, and brood survival (Tepedino and Parker 1983, 1984, Tepedino and Torchio 1989, da Cruz et al. 2006). Much of the experimental research on the effect of nest hole size has been done on members of the family Megachilidae. *Osmia marginata* prefers nests of larger diameter even though they produce higher rates of developmental failure; these larger diameter nests also produce more female offspring (Tepedino and Parker 1983). *Osmia lignaria* and *Hoplitis fulgida* produce larger offspring in nests with larger diameters (Tepedino and Parker 1984, Tepedino and Torchio 1989).

Nesting experiments involving multiple factors (i.e. nest type and microclimate) are more difficult to find. An elegant experiment conducted on the ground nesting bee *Dieunomia triangulifera* examined the interplay of nest site preferences for soil texture and moisture levels (Wuellner 1999). Wuellner discovered that *D. triangulifera* preferred to nest in soils that were compact and moist, with irregular surfaces that received sun. A study of the vespid *Angiopolybia pallens* illustrated that nests at a certain height and diameter that received partial shade were preferred (da Cruz et al. 2006). For some species, such as *Stizus continuus* (Crabronidae) that prefer to nest in aggregations, it is the presence of conspecifics at a site that make it a good choice (Polidori et al. 2008). In reality there are likely many factors that would influence the perfect nesting experience. It would represent a combination of the ideal site, with the ideal substrate, within an

acceptable distance of necessary resources, where interactions with predators and parasites can be avoided.

Nest site selection in Niagara *Ceratina*

Ceratina dupla and *C. calcarata* are common twig-nesting carpenter bees with very similar sympatric distributions encompassing most of eastern North America (Michener 2007). Both species are univoltine and construct one nest per year (Grothaus 1962, Kislow 1976, Rehan and Richards 2010; Chapter 1). A foundress is nest loyal, and once she has provisioned all of her brood she remains at the nest entrance and guards them until their emergence as adults (Rau 1928; Kislow 1976; Rehan and Richards in press, Chapter 1). Females only live for one year, meaning that nest site selection takes place only once for each female, and the offspring reared from that nest represent that female's entire reproductive output for her life time.

Ceratina calcarata nests have been collected from raspberry (*Rubus sp.*), sumac (*Rhus sp.*) and rose (*Rosa sp.*) in Indiana (Grothaus 1962, Johnson 1988), sumac (*Rhus sp.*) in Missouri (Rau 1928), and plume grass (*Erianthus sp.*) in Georgia (Kislow 1976). *Ceratina dupla* has been collected from raspberry (*Rubus sp.*), rose (*Rosa sp.*) and sumac (*Rhus sp.*) in Indiana (Grothaus 1962). In the Niagara Region, *C. dupla* and *C. calcarata* commonly nest in wild raspberry (*Rubus strigosa*), staghorn sumac (*Rhus typhina*), and common teasel (*Dipsacus fullonum*). Plants such as raspberry and sumac are usually located at wood margins which provide shade. The plants themselves are also self shading due to their structure. Teasel provides only one possible nest site per plant unlike

raspberry and staghorn sumac, and experiences a very different microclimate than raspberry or sumac as it is located in full sun, yet all plants are used as nests.

The objectives of this chapter were twofold. The first objective was to determine the potential for interspecific competition for nest sites between *Ceratina dupla* and *C. calcarata,* and whether this competition is reduced through niche partitioning, either spatially or temporally. The second objective aimed to investigate the consequences of nest choice of *Ceratina* in the Niagara region by teasing apart the effects of nesting substrate and nest microclimate on fitness components such as maternal quality, clutch size, and parasitism.

These objectives were investigated using an integrative approach combining field observations, nest collections, and a nest choice experiment. Based on the literature on competition and nest site selection, I generated two hypotheses. The Perfect Fit Hypothesis states that each species has a specific site and substrate preference which has fitness advantages for that species alone. This would imply that *C. dupla* and *C. calcarata* are not in competition with one another for the same nesting microhabitat or substrates, as each species has specific preferences. From this hypothesis I would predict that I would find the same nest site and nest substrate preferences in both the passive collections and during the nest choice experiment. The second hypothesis generated was called The Sharing Hypothesis. This states that both species prefer either the same nest microhabitat, nest substrate, or both, but partition the resource either temporally or spatially to reduce interspecific competition. This hypothesis predicts that the results from the passive nest collections would differ from the nest choice experiment, as competition in nature forces both *C. dupla* and *C. calcarata* to partition resources they

both desire. In addition to nest site and substrate choice, I also investigated fitness correlates of these choices such as maternal body size, clutch size, parasitism and developmental rates.

METHODS

This chapter pertains only to *C. dupla* and *C. calcarata* (a) because *C.* near *dupla* sample sizes were too small, and (b) because it may be bivoltine (Chapter 1), meaning total reproductive output cannot be collected from one nest.

Field site descriptions

Microclimate monitoring

Sites for monitoring microclimate were located on the Brock University Campus and the GQNS. Three sunny (open field) sites were located at Brock South and Brock West on the Brock University Campus, and at the Pond site at GQNS. Three shady sites were located in raspberry patches located in the Brock North/South site, the wood margin of the Ropes Course site (Brock University), and in a raspberry patch near the pond at the Glenridge Quarry Naturalization Site (Figure 1.2).

2008 nest collections

Nest collections were conducted on the Brock University campus, at the Glenridge Quarry Naturalization Site (GQNS), and old abandoned fields on Glenridge Avenue in St. Catharines, Ontario, Canada (Figure 1.2). The Brock University campus

contained several old fields replete with teasel, as well as two raspberry patches and two sumac stands. The GQNS was similar in that it had several large open fields containing teasel as well as two raspberry patches and several areas where sumac was present. The abandoned fields along Glenridge Ave. were used for teasel collections only.

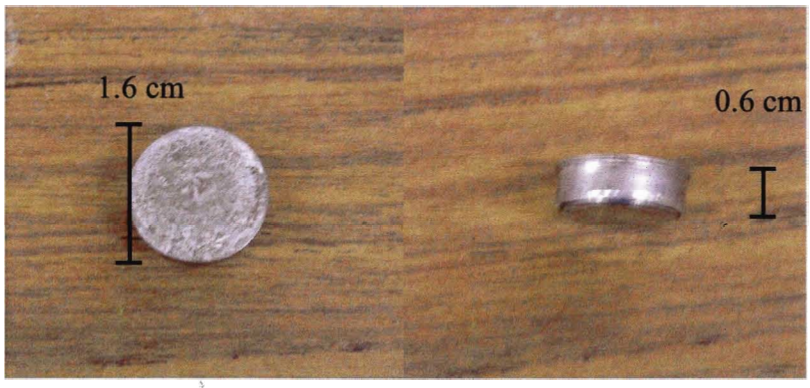
2009 experimental sites

Experimental nest sites in 2009 were all located on the Brock University Campus and chosen based on the fact that they had been good *Ceratina* collecting sites in 2008. The teasel experimental site was located at Brock North, the raspberry site was located in the raspberry patch between Brock North and Brock South, and the sumac site was located in the sumac stand next to the Walker Complex, near the Ropes Course (Figure 1.2).

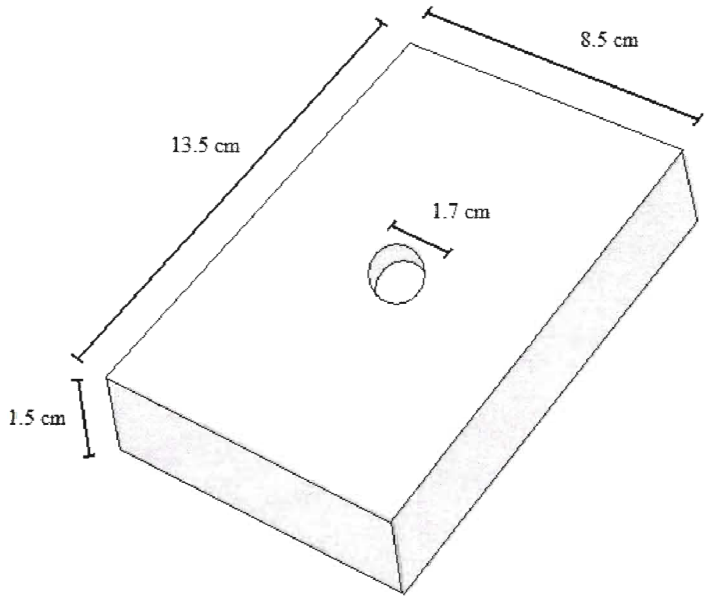
Microclimate differences at sunny and shady nesting sites

While raspberry, teasel and sumac all have pithy stems that can be excavated by female bees to use as nests, they are otherwise quite different. Raspberry and sumac are typically found at shaded wood margins and are both perennial shrubs with many branches that provide potential nest sites. Teasel is a biennial weed that grows in sunny, open, abandoned field settings. It spends its first year as a low profile, broad leaved weed and in its second summer produces a single stalk that grows perpendicular to the ground up to several feet in height. A teasel plant provides only a single potential nest site per plant.

a)



b)



a)

Figure 2.1 a) Top and side view of Ibutton used for taking temperature readings. b) Diagram of wood block used to house Ibuttons in the field.

In order to investigate possible microclimate temperature differences between the two dominant nesting microhabitats of open fields (teasel) and wood margins (raspberry and sumac), small data logging devices that recorded ambient temperature were used (Figure 2.1a). These were approximately the size of a small battery and can be programmed to record ambient temperatures at set times. Each data logger was inlaid in a piece of wood and then covered with masking tape for protection (Figure 2.1b). From 1 April 2008 to 30 September, two data loggers were placed at each of the six monitoring sites (three in open fields and three at wood margins) and synchronized temperature readings were taken every 30 minutes. Every two weeks one data logger from each of the six sites was collected for data downloading and replaced with a new one. Temperature readings along with the time and date were downloaded and recorded. Readings from paired data loggers were compared to ensure that temperature recordings were equivalent and that all data loggers were functional.

Microhabitat and substrate collections in nature

Ceratina from 2008 were collected from nests that had already been initiated by adult bees. For detailed information on nest collections, nest classification, brood rearing, and measurements from 2008 please see methods from Chapter 1.

Experimental test of microhabitat and substrate preferences

While passive nest collections were used in 2008 to represent nest site choices, it was impossible to equalize collecting effort between substrates and therefore I could not

assess actual preferences. In 2009, I designed an experiment that would allow me to equalize sampling effort across species and substrate by offering bees equal numbers of potential nest sites in different microclimates and nesting substrates. The nest choice experiment in 2009 also allowed for the separation of substrate versus site preferences.

A schematic diagram of the experimental design can be seen in Figure 2.2. Three experimental sites were established from 13-17 April 2009, one in an open field (Brock West) where teasel (TS) was present, one in a raspberry patch (RS) between Brock North and Brock South, and one in a stand of staghorn sumac (SS), near the Walker Complex at Brock University. The teasel site received full sun while the raspberry and sumac sites were located at wood margins in the shade.

Ninety twigs each of teasel, raspberry and sumac were collected from the surrounding area and brought back to the lab. Twig lengths were approximately 30-50 cm and twig diameters were 4-7 mm, representing the variation that bees would normally encounter. At each site (TS, RS, and SS), 30 randomly selected twigs of each plant species were set out as nesting substrates for a total of 90 twigs per site. Each twig was securely fastened with masking tape to a 30 cm piece of bamboo stake that had been driven into the ground, similar to the style used by McIntosh (1996).

Twigs were arranged in a grid pattern at all sites. Shape and size of sites dictated the distance between twigs, but an effort was made to replicate the twig densities that bees would naturally encounter in each site. The teasel site was laid out in a 10 x 9 grid with 40 cm between each nest. The raspberry site was also a 10 x 9 grid, however the nests here were placed in closer proximity to one another at a distance of 20 cm. This was done due to the smaller size of the raspberry patch, and also because nests located in

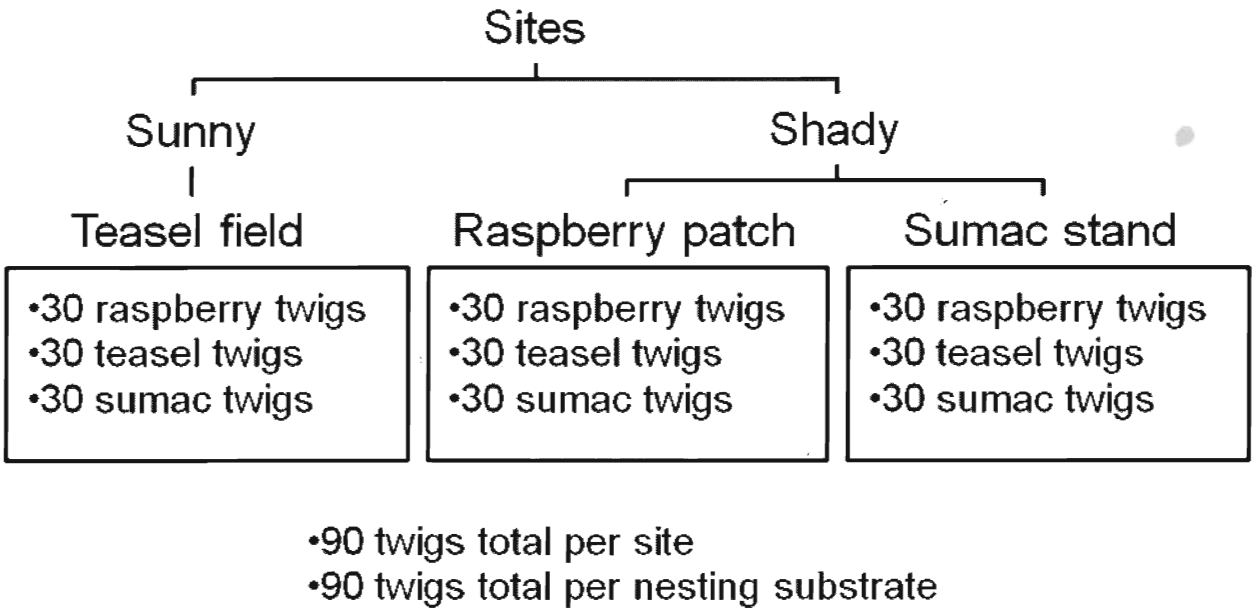


Figure 2.2. Schematic diagram of experimental design for 2009 nest choice experiment. All possible nest substrates (twigs of various species), where attached to bamboo stakes that had been driven into the ground.

raspberry are typically at higher densities than in open fields where teasel is located. The sumac site was narrower but longer than the raspberry and teasel sites and arranged in a grid of 15 x 6. Twigs at this site were 30 cm apart.

All sites were visited twice a week, and each twig was examined to detect whether a female had started a nest. Nest founding could be detected by the appearance of a small hole in the exposed pith of the raspberry, sumac and teasel twigs. Often debris could be seen as the female pushed the recently excavated pith out of the nest entrance. All occupied nests were collected from the field during the week of 13 July 2009, once nest founding had ceased. Nests were brought back to the lab, chilled to anaesthetize the bees inside, and split open to assess the contents. Foundresses were weighed and measured on the day of collection. All immature brood were reared using the same methods as described in Chapter 1.

Data analysis

All data were analyzed using SAS 9.1. Microclimate data were assessed using the Kolmogorov-Smirnov test for distributions, while nest occupancy data were assessed using G tests for goodness of fit in PROC FREQ. Analysis of variance was conducted with PROC GLM. Comparisons of microhabitat and substrate, variation in maternal body size, clutch size and live brood from 2008 nest collections was compared using a one-way ANOVA test among sites for each species individually. In 2009 comparisons could only be made between the nesting substrates due to the fact that almost all nests were founded in the sun (teasel site). These comparisons were also accomplished using

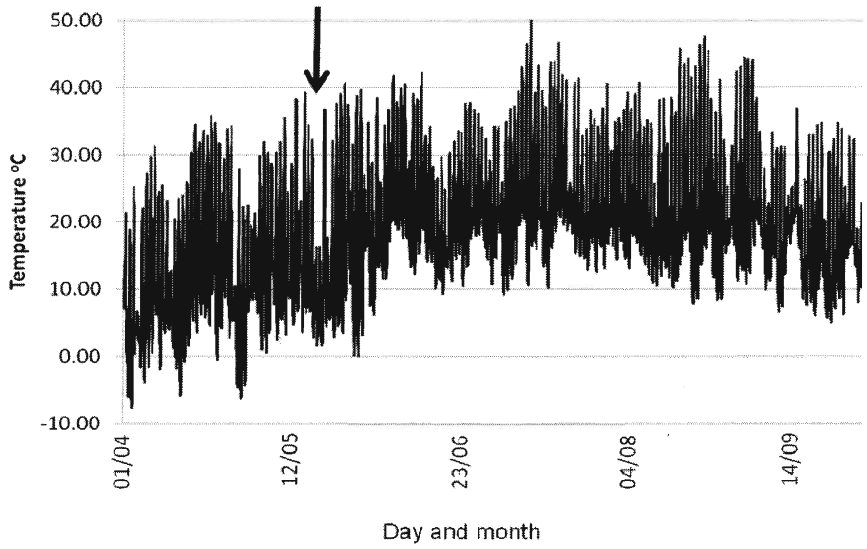
one-way ANOVAs comparing nesting substrates. Clutch size was calculated as the total number of provisioned cells per nest. Number of live brood was calculated as the number of provisioned cells that contained live, unparasitized brood. Only full brood nests were used for calculations of clutch size and live brood, to ensure that these variables were based on complete clutches.

RESULTS

Microclimate data

The mean open field and wood margin temperature traces can be seen in Figure 2.3. The temperature distributions of the sunny teasel site and the shady raspberry site were significantly different (Kolmogorov-Smirnov; $D=0.16$, $KSa=10.37$, $P<0.0001$). Temperatures in the open field were quite variable and on average were higher, while the temperatures at the wood margin sites were much less variable and on average lower (Figure 2.3). Both sunny and shady sites experienced similar low temperatures, but the sunny sites experienced greater high temperatures than the shady sites. In early spring, both sites were fairly exposed, however by the end of May foliage development provided shade for all plants along the wood margin. Although temperatures recorded by data loggers were often higher than the ambient air temperature, especially in sunny sites, all data loggers were treated in the same manner so differences between the sites reflect different patterns of insolation.

a) open field (sunny)



b) wood margin (shady)

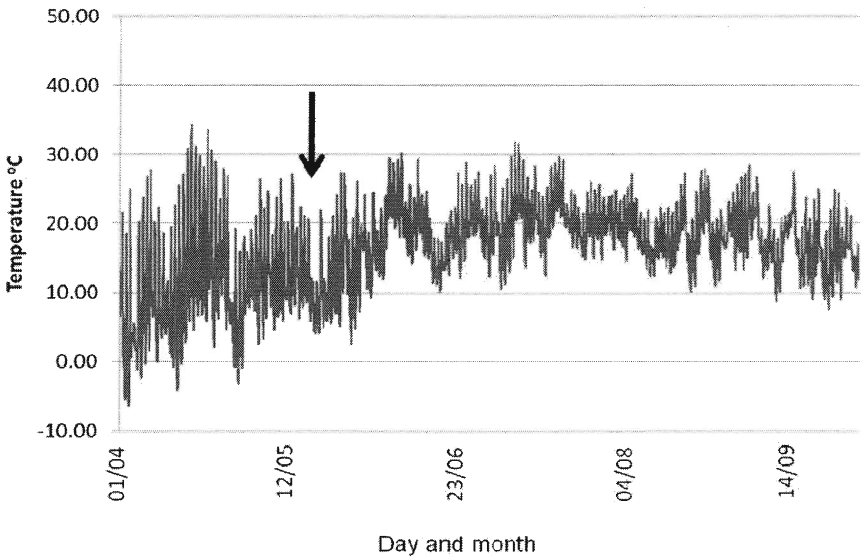


Figure 2.3. Average temperatures recorded by data loggers in (a) all open field sites combined and (b) all wood margin sites combined. Readings were taken every 30 min from 1 April to 30 September 2008. Note that as the season progressed, the variation in temperature at the wood margin sites became much smaller than that of the open field sites which corresponds with foliage development (arrows).

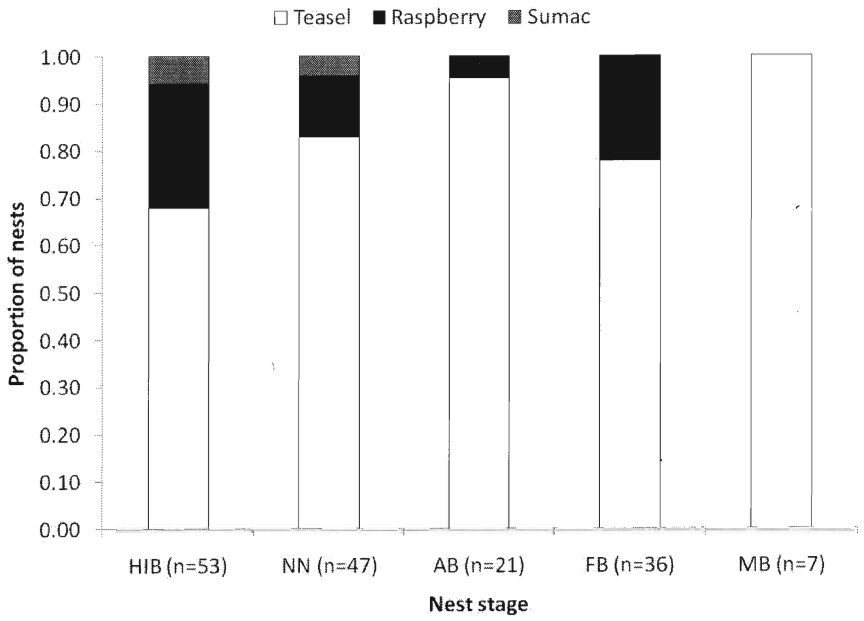
Nesting substrate preferences

2008 nest collections

In 2008, 401 *Ceratina* nests were collected from teasel (found in open fields), raspberry (at wood margins) or sumac (at wood margins). Seasonal changes in nest substrate usage are indicated by differences in substrate use among nest stages (Figure 2.4). Both species used sumac more often as a place to overwinter (hibernacula) than they did for actively nesting (*C. dupla* G=6.08, d.f.=1, P=0.04, *C. calcarata* G=17. 00, d.f.=1, P=0.0002)(Figure 2.4). A few *C. dupla* hibernacula were collected from sumac, however no *C. dupla* females were ever found to actively provision in sumac twigs (Figure 2.4).

Of 64 *C. dupla* nests, more than 80% were in teasel with the remaining fraction being collected from raspberry (Figure 2.5a). No active *C. dupla* nests were ever found in sumac. *Ceratina calcarata* nests were occasionally collected from teasel (36%), but the majority were collected from raspberry (46%) with some in sumac (18%) (Figure 2.5a). Only active, full and mature brood nests were used for this analysis, as these females had made the decision to lay eggs in these nests, which was not true for hibernacula, and not necessarily true for new nests.

a) *C. dupla*



b) *C. calcarata*

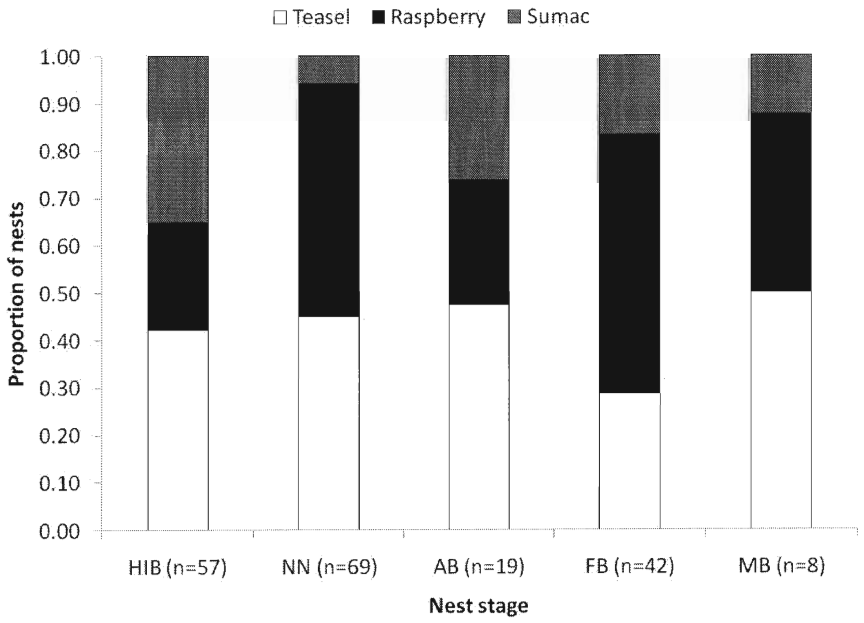


Figure 2.4. Substrate use for each nest type from 2008 passive nest collections for (a) *C. dupla* and (b) *C. calcarata.* HIB=hibernacula (spring only), NN=new nest, AB=active brood, FB=full brood, and MB= mature brood.

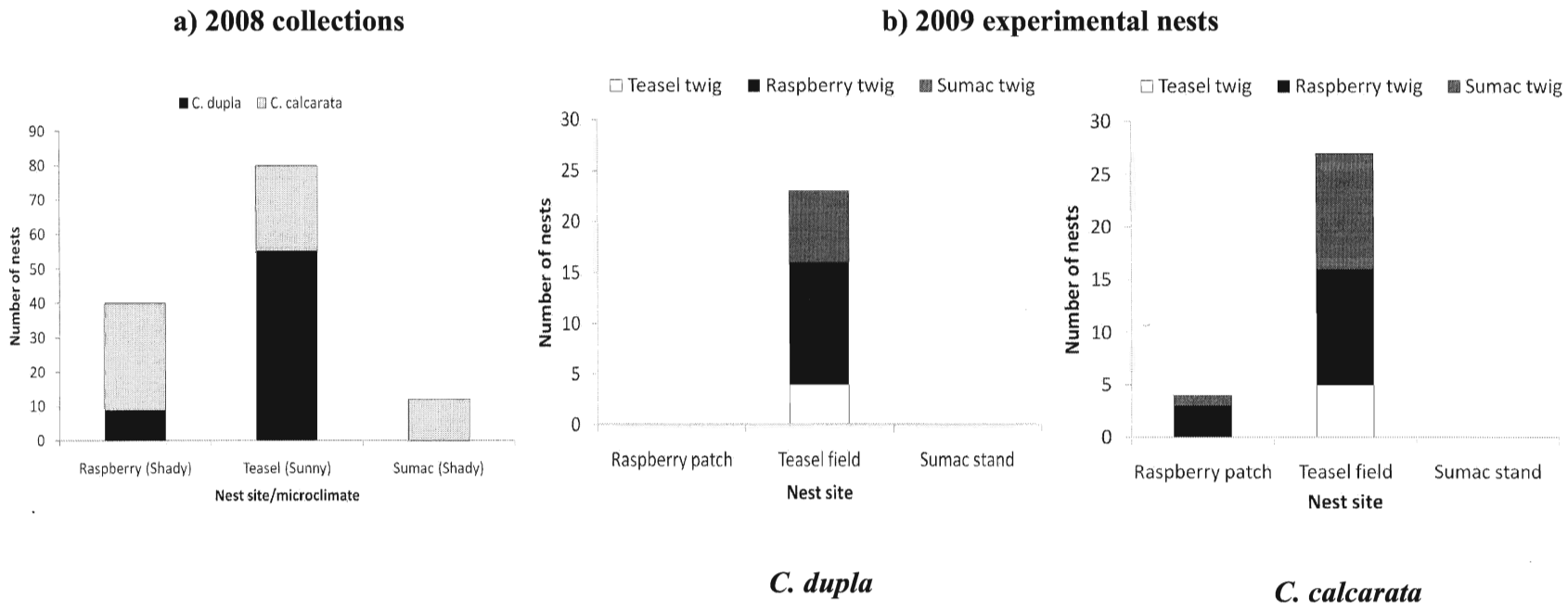


Figure 2.5. Substrate use by *C. dupla* and *C. calcarata* nest collections for (a) active, full and mature brood nests from 2008 nest collections, and (b) active and full brood nests 2009 experimental nest collections. Nests in 2008 could only be collected where they naturally occurred i.e. teasel in the sun, or raspberry and sumac in the shade, while in 2009 all nest substrates were available in all microclimates.

2009 experimental nests

Of the 270 twigs available, 99 (37%) were occupied by some type of arthropod at the time of collection (Table 2.1). *Ceratina* were the most common inhabitants, occupying 58 nests in total (21% of all available twigs, including males). Twigs were also occupied by ants (Formicidae), earwigs (Forficulidae), bees from the family Megachilidae, one bee from the family Colletidae (genus *Hylaeus*), wasps (Crabronidae), caterpillars (Lepidoptera), and an unknown aculeate wasp species (Hymenoptera). In 22 twigs, there was an empty nest, implying that some type of twig-nesting arthropod had begun to excavate a burrow and then abandoned it for some reason.

In 2009, *C. dupla* nested much more often at the teasel site (21 nests), and did not nest at the raspberry or sumac site ($G=46.42$, d.f.=2, $P<0.0001$) (Figure 2.5b). *C. calcarata* also nested most commonly in the teasel site (28 nests), occasionally at the raspberry site (4 nests) and never at the sumac site ($G=44.27$, d.f.=2, $P<0.0001$) (Figure 2.5b). At the sumac site, seven nests were initiated (five in raspberry twigs, one in a teasel twig and one in a sumac twig), but were abandoned before provisioning began. The only *Ceratina* collected from the sumac site was one *C. calcarata* male found in a raspberry twig.

Preferences for nesting substrate were not as clear cut as site preferences. Both *Ceratina* species founded nests in all three substrates (Figure 2.5b). *Ceratina calcarata* and *C. dupla* founded the most nests in raspberry twigs (11 for each species), fewer in sumac (12 and 7 respectively), and the fewest nests in teasel (5 and 3 respectively) (Figure 2.5b). While differences in substrate preference were not statistically significant when each species was analyzed individually

Table 2.1. Nest occupation by all arthropods found in 2009 experimental nests. Abandoned nests have been included in the table but not in the occupancy calculations. Significantly more twigs at the teasel site were occupied ($G = 74.20$, d.f.=2, $P<0.0001$), however there was no difference in occupation rates between the three different twig species ($G=5.45$, d.f.=2, $P=0.07$.).

Site	**Substrate**			
	Raspberry	Teasel	Sumac	**Site total**
Raspberry	3 *Ceratina* ♀ 2 *Ceratina* ♂ 1 Colletidae 1 Forficulidae 1 Formicidae 1 Megachilidae	1 Crabronidae 1 Forficulidae *3 abandoned*	1 *Ceratina* ♀ 2 Aculeata sp.1 1 Crabronidae 1 Forficulidae 2 Formicidae *4 abandoned*	
***Ceratina* ♀ occupancy**	**3 (10%)**		**1 (3%)**	**4/90 (4%)**
Total occupancy	**9 (30%)**	**2 (7%)**	**7 (23%)**	**18/90 (20%)**
Teasel	23 *Ceratina* ♀ 1 Megachilidae *1 abandoned*	9 *Ceratina* ♀ 5 Aculeata sp. 1 1 Lepidoptera 2 Megachilidae *1 abandoned*	18 *Ceratina* ♀ 1 *Ceratina* ♂ 1 Aculeata sp. 1 1 Formicidae 1 Lepidoptera 1 Megachilidae *4 abandoned*	
***Ceratina* ♀ occupancy**	**23 (77%)**	**9 (30%)**	**18 (63%)**	**50/90 (56%)**
Total occupancy	**24 (80%)**	**17 (57%)**	**23 (77%)**	**64/90 (71%)**
Sumac	1 *Ceratina* ♂ 6 Aculeata sp. 1 *5 abandoned*	1 Aculeata sp.1 2 Crabronidae 4 Forficulidae *1 abandoned*	1 Aculeata sp. 1 *1 abandoned*	
***Ceratina* ♀ occupancy**	**0 (0%)**	**0 (0%)**	**0 (0%)**	**0/90 (0%)**
Total occupancy	**7 (23%)**	**7 (23%)**	**1 (3%)**	**16/90 (18%)**
Substrate total	**41/90 (46%)**	**26/90 (29%)**	**32/90 (21%)**	**99/270 (37%)**

(*C. dupla:* G=4.86, d.f.=2, P=0.09, *C. calcarata:* G=5.18, d.f.=2, P=0.08), they were significant when both species were pooled, showing that *Ceratina* nest in teasel less often than raspberry or sumac (G=9.56, d.f.=2, P=0.008, Table 2.2).

Correlates of nest site selection

Maternal body size

Head width was used as the measurement for maternal body size for both *C. dupla* and *C. calcarata.* Hibernacula and new nests were excluded from this analysis. In 2008 *Ceratina dupla* females did not differ in size between teasel (sunny) and raspberry (shady) nests (none nested in sumac) (ANOVA $F_{(1,96)}$=1.87, n.s., Table 2.2). *Ceratina calcarata* females also did not differ in body size among raspberry, teasel and sumac nests (ANOVA $F_{(2,72)}$=1.51, n.s., Table 2.2)

A similar result was found in the experimental nests in 2009. As no *C. dupla* nests were collected from the raspberry site a comparison could only be made among substrates in the teasel site. *Ceratina dupla* females nesting in raspberry, teasel or sumac twigs at the teasel site were not different in body size (ANOVA, $F_{(2,15)}$=1.80, n.s.; Table 2.3). As so few *C. calcarata* females chose to nest at the raspberry site comparisons between site could not be made. *Ceratina calcarata* females were not different in body size between nesting substrates (ANOVA, $F_{(2,29)}$=1.17, n.s.)

Table 2.2. Mean female head width ± SD (n) for *C. dupla* and *C. calcarata* from 2008 nest collections. Hibernacula and new nests were not included in analysis.

	Substrate and microclimate			
Species	Raspberry (Shade)	Teasel (Sun)	Sumac (Shade)	**Species mean**
C. dupla	1.87±0.17 (22)	1.93±0.16 (76)	-	1.91 ±0.16 (98)
C. calcarata	2.01±0.11 (32)	1.95±0.21 (32)	1.94±0.11 (11)	1.97±0.17 (75)
Mean	1.96±0.15 (54)	1.93±0.18 (108)	1.94±0.11 (11)	

Table 2.3. Mean head width (mm) ± SD (n) for a) *C. dupla* and b) *C. calcarata* from 2009 nest choice experiment. Only full brood nests were used for head width analysis. No nests were founded in sumac and were therefore unavailable for analysis.

a) *C. dupla*

	Substrate			
Site	Raspberry	Teasel	Sumac	**Site mean**
Raspberry	-	-	-	-
Teasel	1.92±0.20 (10)	2.12±0.19 (3)	2.05± 0.19 (5)	1.99± 0.2 (18)
Sumac	-	-	-	-
Substrate mean	1.92±0.20 (10)	2.12±0.19 (3)	2.05± 0.19 (5)	1.99± 0.2 (18)

b) *C. calcarata*

	Substrate			
Site	Raspberry	Teasel	Sumac	**Site mean**
Raspberry	1.87±0.14 (2)	-	1.82 (1)	1.85±0.10 (3)
Teasel	1.91±0.16 (11)	1.99±0.11 (5)	1.98± 0.12(11)	1.96±0.14 (27)
Sumac	-	-	-	-
Substrate mean	1.90±0.15 (13)	1.99±0.11 (5)	1.97±0.13 (12)	1.95±0.14 (30)

Offspring body size

Offspring body size for *C. dupla* in 2008 did not differ between the sunny teasel nests and the shady raspberry nests (Table 2.4a). However, those *C. calcarata* individuals that were reared from sumac nests were significantly smaller than those reared from teasel nests in 2008 (Table 2.4a). For 2009, comparisons of offspring body size were among substrates at the teasel site only. *Ceratina dupla* offspring did not differ in body sizes among the raspberry, teasel and sumac twigs in the sunny teasel site, nor did *C. calcarata* offspring (Table 2.4b).

Clutch size and live brood

Ceratina dupla mean clutch sizes were very similar in teasel (sun) and raspberry (shade) in 2008 (ANOVA, $F_{(1,35)}$=0.04, n.s., Table 2.5a). No active *C. dupla* nests were collected from sumac. *Ceratina dupla* live brood sizes also showed no difference between the teasel (sun) and the raspberry (shade) sites (ANOVA $F_{(1,35)}$=0.70, n.s., Table 2.4a). *Ceratina calcarata* nests had significantly larger clutch sizes in teasel (sun) than in sumac (shade), with moderate clutch sizes in raspberry (shade) in 2008 (ANOVA $F_{(2,36)}$=3.67, P=0.04, Table 2.5b). Live brood sizes also differed for *C. calcarata* in 2008. The number of live brood from *Ceratina calcarata* nests in teasel was significantly greater than in raspberry or sumac ($F_{(2,36)}$=12.30, P<0.0001, Table 2.5b). This indicates that in 2008 *C. calcarata* nests in teasel were larger and experienced less parasitism and developmental failure than those in raspberry and sumac.

Table 2.4. Mean offspring body size (mg) for *C. dupla* and *C. calcarata* from (a) nest collections in 2008, and (b) 2009 experimental nests from the sunny teasel site only.

a) 2008 nest collections

	Substrate and microclimate			
Species	Raspberry (Shade)	Teasel (Sun)	Sumac (Shade)	**Statistical test**
C. dupla	8.74±2.32 (63)	8.95±3.30 (225)	-	$F_{(1,286)}$=0.22, n.s.
C. calcarata	9.69±2.76[a] (92)	8.94±2.57 (79)[ab]	7.80±1.86[b] (14)	**$F_{(2, 182)}$=3.96, P=0.02**

b) 2009 experimental nests

	Sunny (teasel) site only			
Species	Raspberry twig	Teasel twig	Sumac twig	**Statistical test**
C. dupla	11.24±2.85 (7)	14.93±3.31 (6)	11.95±6.13 (7)	$F_{(2,17)}$=1.25, n.s.
C. calcarata	12.97±1.10 (3)	-	12.32±4.93 (11)	$F_{(1, 12)}$=0.06, n.s.

Table 2.5. The effect of nesting substrate on brood productivity for *C. dupla* and *C. calcarata* from 2008 nest collections. Only full brood nests were used for these analyses. Nests with different letters are significantly different from one another.

a) *C. dupla*

	Substrate and microclimate			
	Raspberry (Shade)	Teasel (Sun)	Sumac (Shade)	**Mean**
Clutch size ± SD (n)	11.75±4.0 (8)	11.41±4.2 (29)	-	11.48 ±4.1 (37)
Live brood ± SD (n)	8.6±4.0 (8)	7.1±4.6 (29)	-	7.5 ±4.5 (37)

b) *C. calcarata*

	Substrate and microclimate			
	Raspberry (Shade)	Teasel (Sun)	Sumac (Shade)	**Mean**
Clutch size ± SD (n)	7.4±3.6 (25) ^{ab}	10.57±4.9 (7) ^{a}	5.14±3.2 (7) ^{b}	7.56±4.0 (39)
Live brood ± SD (n)	3.6±2.6 (25) ^{b}	6.69±3.2 (7) ^{a}	1.0±1.2 (7) ^{b}	4.0±3.2 (39)

The average clutch size of *C. dupla* from nests in 2009 was the same in all substrates at the teasel site (ANOVA $F_{(2,16)}$=0.19, n.s., Table 2.6a). Live brood sizes at the teasel site were also not different between substrates for *C. dupla* ($F_{(2,16)}$=0.11, n.s., Table 2.6a). Comparisons of clutch size and live brood for *C. calcarata* in 2009 revealed similar results to those seen in *C. dupla* (Table 2.6b). Clutch size did not differ among nests founded in raspberry, teasel and sumac twigs ($F_{(2,22)}$=1.20, n.s.) nor did live brood sizes ($F_{(2,22)}$=3.04, n.s.; Table 2.6b).

Parasitism

Parasitism rates for *Ceratina dupla* females in 2008 did not differ among nests in teasel (sunny) or raspberry (shady) (Table 2.7a). This was true both when looking at the number of nests that contained at least one parasite (prevalence), as well as the total number of available individuals parasitized (virulence) (Table 2.7a). A different pattern occurred in *C. calcarata* (Table 2.7b). Significantly more raspberry nests (21/24, 84%) contained at least one parasite, as compared to 20% (2/10) of nests in sumac, and 57% (4/7) of nests in raspberry (Table 2.7b). *Ceratina calcarata* nests laid in raspberry also had the most individuals parasitized, followed by sumac and then teasel (Table 2.7b).

Parasitism rates among the three different nest substrates in the sunny (teasel) site in 2009 show no difference in either prevalence or virulence for *C. dupla* or *C. calcarata* (Table 2.8).

Table 2.6. Mean clutch sizes and live brood for a) *C. dupla* and b) *C. calcarata* from 2009 nest choice experiment. Clutch size is shown in normal font while live brood is shown in italics. Sample size is the same for clutch size and live brood calculations.

a) *C. dupla*

	Substrate ± SD (n)			
Site ± SD (n)	Raspberry	Teasel	Sumac	**Site mean**
Raspberry	-	-	-	-
Teasel	11.8±3.9 (9) *7.6± 3.4*	13.0±6.1 (3) *9.7± 2.0*	13.2±4.9 (5) *7.9± 3.8*	12.4±4.3 (17) *7.9± 4.0*
Sumac	-	-	-	-
Substrate mean	11.8±3.9 (9) *7.6± 3.4*	13.0±6.1 (3) *9.7± 2.0*	13.2±4.9 (5) *7.9± 3.8*	12.4±4.3 (17) *7.9± 4.0*

b) *C. calcarata*

	Substrate ± SD (n)			
Site ± SD (n)	Raspberry	Teasel	Sumac	**Site total**
Raspberry	6.0 ± 0 (2) *3.5± 2.1*	-	-	6.0 ± 0 (2) *3.5* ± 2.1
Teasel	8.9±1.8 (8) *6.4± 2.3*	9.0±3.6 (3) *2.0± 2.0*	7.0±2.5 (12) *5.2± 2.3*	7.9± 2.5 (23) *5.2± 2.6*
Sumac	-	-	-	-
Substrate mean	8.3±2.0 (10) *5.8± 2.5*	9.0±3.6 (3) *2.0± 2.0*	7.0±2.5 (12) *5.2± 2.3*	7.8±2.5 (25) *5.0*± 2.6

Table 2.7. Parasitism rates in *Ceratina* nests from 2008 passive nest collections for a) *C. dupla* and b) *C. calcarata.* Prevalence is defined as the number of nests containing at least one parasite, while virulence is the total number of individuals parasitized divided by the total number available.

a) *C. dupla*

	Substrate and microclimate			
Species	Raspberry (Shade)	Teasel (Sun)	Sumac (Shade)	**Statistical Test**
Prevalence	7/7 (100%)	19/31 (61%)	-	n.s., Fisher's exact
Virulence	18/91 (20%)	93/323 (29%)	-	X^2=0.33, d.f.=1, n.s.

b) *C. calcarata*

	Substrate and microclimate			
Species	Raspberry (Shade)	Teasel (Sun)	Sumac (Shade)	**Statistical Test**
Prevalence	21/25 (84%)	2/10 (20%)	4/7 (57%)	**P=0.007, Fisher's exact**
Virulence	84/180 (47%)	10/71 (14%)	15/33 (45%)	**X^2=23.65, d.f.=2, P<0.0001**

Table 2.8. Parasitism rates in *Ceratina* nests from 2009 experimental nests in the teasel site only for a) *C. dupla* and b) *C. calcarata.*

a) *C. dupla*

	Teasel site only			
Species	Raspberry twig	Teasel twig	Sumac twig	**Statistical Test**
Prevalence	9/11 (82%)	2/3 (66%)	5/7 (71%)	n.s., Fisher's exact
Virulence	28/119 (24%)	5/22 (23%)	14/93 (15%)	X^2=2.51, d.f.=2, n.s.

b) *C. calcarata*

	Teasel site only			
Species	Raspberry twig	Teasel twig	Sumac twig	**Statistical Test**
Prevalence	6/11 (55%)	4/5 (80%)	7/12 (58%)	n.s. Fisher's exact
Virulence	11/89 (12%)	13/45 (29%)	17/88 (19%)	X^2=4.36, d.f.=2, n.s.

Developmental rates

In 2008, *Ceratina dupla* brood raised in raspberry had significantly faster developmental rates than those developing in teasel when reared in the lab (Table 2.8a). The same pattern was true for *C. calcarata,* showing that individuals laid in raspberry developed faster in the lab than those in teasel (Table 2.8a).

As only three offspring were reared from nests in the raspberry site in 2009, comparisons were made for substrates within the teasel site. Developmental rates for *C. dupla* did not differ among raspberry, teasel and sumac twigs in the teasel site Table 2.8b). The same pattern was true for *C. calcarata.* Individuals nesting in the three different substrates in the teasel site did not develop at different rates (Table 2.8b). This means that the differences seen in developmental rates were due to the microhabitat and not the nest substrate.

DISCUSSION

Ceratina nest preferences

Combining the data from the nest collections in 2008 with the data from the nest choice experiment in 2009 is a powerful way to tease apart the difference between bees' nesting choices versus their actual preferences. In nature both *C. dupla* and *C. calcarata* can only nest in what is available to them- they have to make choices based on availability, and this is what was assessed in 2008. The availability of all nest substrate types in all microclimates in 2009 allowed for the assessment of preferences for both microclimate and substrate.

Table 2.9. Developmental rates expressed as stages passed per day for *C. dupla* and *C. calcarata* in (a) raspberry and teasel from 2008 passive nest collections and (b) from the teasel site only in the 2009 passive nest collections.

a) 2008 nest collections

	Developmental stages per day ± SD (n)		
Species	Raspberry (Shade)	Teasel (Sun)	Kruskal Wallis
C. dupla	0.53 ± 0.10 (66)	0.48 ± 0.11 (170)	**H=19.78, d.f.=1, P<0.0001**
C. calcarata	0.57 ± 0.21 (81)	0.49 ± 0.13 (72)	**H=15.23, d.f.=1, P<0.0001**

b) 2009 experimental nests (teasel site only)

	Developmental stages per day ± SD (n)			
Species	Raspberry twig	Teasel twig	Sumac twig	Kruskal Wallis
C. dupla	0.49 ± 0.15 (52)	0.44 ± 0.05 (24)	0.42±0.05 (35)	H=2.62, d.f.=2, n.s
C. calcarata	0.42 ± 0.04 (37)	0.36 ± 0 (2)	0.42±0.05 (32)	H=2.10, d.f.=2, n.s.

Both *C. dupla* and *C. calcarata* were found to nest in three species of plants (raspberry, teasel and sumac) which grow in two different microclimates (full sun and shade). Each plant species has its corresponding microhabitat; teasel grows in full sun while raspberry and sumac are found in more shaded areas. Nest collections from 2008 show that *C. dupla* and *C. calcarata* do not nest in each plant species equally. *Ceratina dupla* was collected most frequently from teasel (sun), rarely in raspberry (shade) and never in sumac (shade), whereas *C. calcarata* was collected most often in raspberry (shade) and sumac (shade) and occasionally in teasel (sun).

The nest choice experiment in 2009 allowed for untangling substrate vs. microhabitat preferences for both species. Both species had the option of nesting in any of the three plant species in any of the three nest sites. By far the strongest result was seen in the choices made for nest site. Both *Ceratina dupla* and *C. calcarata* overwhelmingly chose to nest in the sunny site (teasel field), rather than the shady sites (raspberry and sumac). In fact only four *Ceratina calcarata* females chose to nest in the shady raspberry site, and no *Ceratina* females of either species were found nesting at the sumac site. Moreover, both species of *Ceratina* nested most often in raspberry and sumac twigs. While both *Ceratina* species greatly preferred to nest at the sunny teasel site, their least preferred substrate was teasel twigs. They also both nested in sumac twigs in the teasel sites, indicating that it is the sumac site and not the substrate that makes it a less desirable option.

This outcome is congruent with The Sharing Hypothesis which stated that both species would prefer the same nest site, nest substrate, or both, but would partition the resources to reduce interspecific competition. The 2009 experiment showed that given all

options, both *C. dupla* and *C. calcarata* have the same preferences for both microhabitat and substrate, that being raspberry and sumac twigs in the sun. While all nest substrates were available in all microclimates during the nest choice experiment, the option of nesting in teasel in full shade or raspberry and sumac in full sun would be extremely rare in nature. This implies that each bee must make a choice to nest either in the preferred microclimate or in the preferred nest substrate. The 2008 collections show that the majority of *C. dupla* females end up nesting in the preferred microhabitat (sun) and therefore in teasel, while most *C. calcarata* nest in the preferred substrate (raspberry and sumac), and therefore in the shade (Figure 2.6).

Consequences of nest microhabitat and substrate

The decision of where to nest comes with several fitness consequences. A summary of these consequences associated with microclimate and nest substrate can be seen in Table 2.10. Giving the bees the option to choose their own nests during the 2009 choice experiment also allowed them to dictate sample size. What resulted was the overwhelming decision of *Ceratina* to nest in the sunny site (teasel) over the shady sites (raspberry and sumac) as well as raspberry and sumac twigs over teasel twigs. While this does lead to a lack of power for the comparisons, it also reflects the preference of each species for nesting in sunny areas. Thirty twigs of each substrate were available at each site, so the opportunity to nest in each site/substrate scenario was equal for each species. In most cases the results from 2009 with smaller sample sizes also reflect those of 2008 which have much larger sample sizes.

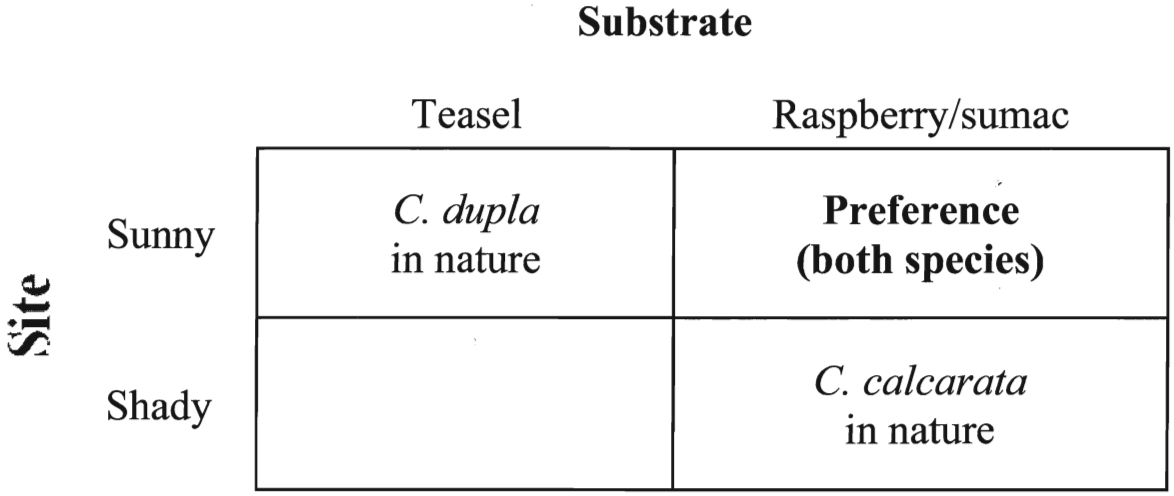


Figure 2.6 Schematic diagram of the site and substrate options available to *Ceratina dupla* and *C. calcarata.* Both species would prefer to nest in raspberry and sumac in the sunny sites, however in nature we find *C. dupla* predominately in the sunny site nesting in teasel twigs, while *C. calcarata* nests in the shade site in raspberry and sumac twig

Table 2.10. Summary of fitness correlates of nest site selection for *C. dupla* and *C. calcarata* based on observational results from 2008 and experimental results from 2009.

	C. dupla		*C. calcarata*	
	Microclimate	**Substrate**	**Microclimate**	**Substrate**
Maternal body size	No effect	No effect	No effect	No effect
Brood body size	No effect	No effect	**Smaller in shade**	No effect
Clutch size	No effect	No effect	**Higher in sun**	No effect
Number of live brood	No effect	No effect	**Higher in sun**	No effect
Parasitism	No effect	No effect	**Higher prevalence and virulence in shade**	No effect
Brood developmental rate	**Upregulated in shade**	No effect	**Upregulated in shade**	No effect

Two interesting patterns emerged when examining fitness correlates of nest microclimate and substrate. First, the substrate itself had no effect on any of the parameters examined. This would explain why the 2009 results showed an extreme preference for sunny microhabitat, and a secondary preference for raspberry and teasel twigs. Second, aside from developmental rates, *C. dupla* appeared not to be nearly as affected by the difference between sunny versus shady microclimates as *C. calcarata.*

The only microclimate parameter affecting both *C. dupla* and *C. calcarata* was that developmental rate was upregulated for those individuals nesting in shade. This only became evident when *C. dupla* and *C. calcarata* offspring from both the shade and the sun were reared in the lab at the same temperature. The 2009 experiment demonstrated that brood reared from the three different plant substrates at the same site produced similar developmental rates, showing that it is microclimate and not substrate that are more important. Poikilotherms raised at warmer temperatures normally develop at faster rates than those at cooler temperatures, a pattern referred to as cogradient variation (Blanckenhorn 1991, Conover and Schultz 1995). Countergradient variation is the opposite: poikilotherms from colder temperatures develop more quickly than those at warmer temperatures, because metabolic rates are up-regulated rather than simply being passive responses to external temperature. Countergradient variation has been observed for species of fish, amphibians, and arthropods (Blanckenhorn 1991, Schultz et al. 1996, Skelly 2004, Marcil et al. 2006). It appears that both *C. dupla* and *C. calcarata* immatures are able to compensate for different conditions they may encounter in nature. The ability to regulate developmental rates for warmer or cooler conditions would be a

huge advantage, allowing *Ceratina* females to nest in a wide range of habitats, as well as in cooler climates, without having much effect on the duration of larval development.

Microclimate affected *C. calcarata* brood body size, clutch size, live brood, and rates of parasitism. The higher clutch sizes attained at the sunny site may have been due to the surrounding plant life as opposed to the sun itself. The sunny nests were located in open fields replete with wildflowers from which females collect pollen. By nesting in the open field in close proximity to pollen resources, females may have been able to provide more and larger provision masses than if they had chosen to nest at wood margins. Distance to resources has been shown to affect the number of total brood cells provisioned as well as the number of brood cells provisioned per day for the megachilids *Megachile rotundata* and *M. apicalis* (Kim 1999, Peterson and Roitberg 2006a, Peterson and Roitberg 2006b).

The higher parasitism rates seen in the shady sites may also have had less to do with microclimate and more to do with the actual plant itself. Both raspberry and sumac are native plants to the Niagara Region while teasel is an introduced species (Rector et al. 2006). Many parasitoid species search for their hosts by seeking out the plant(s) used by their hosts (Vet 1983, Elzen et al. 1986), or by microhabitat they commonly inhabit (Gibson 1990). By nesting in a relatively new substrate, *Ceratina* females nesting in teasel may be outside the search image of their usual parasitoid hosts.

Evidence for competition and resource partitioning

In order to conclude that there is competition for nest sites between *C. dupla* and *C. calcarata* two things are necessary. The first is that both species have the same

resource preferences. This was demonstrated during the 2009 nest choice experiment: both species preferred raspberry and sumac twigs at the sunny site. Also important to demonstrate is that the preferred resource is limiting in nature. Nests in the sunny teasel site in 2009 were very popular, with 57% being occupied by a *Ceratina* female. *Ceratina* were also not the only insects that were found nesting in twigs at the teasel site. If all arthropod species are included the occupation rate at the teasel site increases to a very high 71 %. *Ceratina* species are not only in competition with each other, but also with other twig nesting arthropods that may be a part of the community.

Also interesting is the history and biology of the teasel substrate itself. Wild teasel (*Dipsacus fullonum*) had a relatively recent introduction into North America from Europe. A biennial weed, it was most likely introduced by John Bartram into Pennsylvania in 1728 along with cultivated teasel (*D. sativus*), an obsolete crop plant used to raise the knap in wool (Rector et al. 2006). Teasel plants only become available as nests to *Ceratina* species the spring after the plant has died, making the window of opportunity for nesting in teasel much narrower than that of the other two substrates. The dead teasel stalk is then usually destroyed the following winter.

The fact that *Ceratina* are nesting in a recently introduced, non-native species, with relatively short nesting availability at all indicates that nest sites may have been limiting prior to its introduction. Once teasel became available bees would face a novel nest site selection decision. There were now enough nest sites available but each one has its advantages and disadvantages. Teasel grows in preferred microhabitat which received the most sunlight, but it is a less desirable nesting substrate. Raspberry and sumac are more desirable substrates (and possibly more familiar), but do not receive nearly as much

sun as teasel nests. It appears that *Ceratina dupla* and *C. calcarata* have partitioned these resources, with *C. dupla* nesting in the preferred sunny microclimate (and therefore in the least preferred substrate), while *C. calcarata* nests in raspberry and sumac, the preferred substrate (and therefore in the least preferred microhabitat.)

Niche partitioning in the subgenus *Ceratinidia*

Ceratina in the Niagara Region are not unique in having multiple, similar species coexisting in the same community. A remarkable parallel to the *C. calcarata/C. dupla* species pair comes from a pair of *Ceratina (Ceratinidia)*. *Ceratina japonica* and *C. flavipes* are both common in Japan and southern China (Yasumatsu and Hirashima 1969). Females of *C. japonica* and *C. flavipes* are difficult to differentiate from one another while the males can be told apart more easily (Shiokawa 1963b). Superficially occupying similar habitat, initial studies of nest architecture and phenology failed to show any clear species differences (Shiokawa 1963a, Kurihara et al. 1981). However, over the course of several studies, an accumulation of small biological differences were noted. A summary table of these traits is compared in Table 2.11. Just as in the Ontario *Ceratina*, one Japanese species (*C. flavipes*) prefers to nest in open fields while the other (*C. japonica*) prefers nest substrates found at wood margins (Sakagami and Maeta 1977). Like *C. dupla, C. flavipes* migrates between wood margins and open fields indicating that it overwinters in the habitat typical of their sympatric partner (Sakagami and Maeta 1977). Due to this migration away from natal nests, both open field-nesting species must construct new hibernacula, whereas both species nesting at wood margins almost always

Table 2.11. Life history traits highlighting the similarities of the *dupla-calcarata* group and the *flavipes-japonica* group. Nest site crossover implies that species is also found nesting in the common microhabitat of the other species in the subgenus.

	Subgenus and species			
	(Zadontomerus)		*(Ceratinidia)*	
Trait	*C. dupla*	*C. calcarata*	*C. flavipes*	*C. japonica*
Male morphology	Simple to differentiate		Simple to differentiate	
Female morphology	Difficult to differentiate (almost identical)		Difficult to differentiate (almost identical)	
Nest site preference	Sun	Shade	Sun	Shade
Natal nest hibernacula?	Mostly new	Usually yes	New	Usually yes
Hibernacula location	*C. calcarata* habitat	Own habitat	*C. japonica* habitat	Own habitat
Clutch size	11.5±4.3	7.6±4.0	~10.4	~6.5

reuse their natal nests as hibernacula (Sakagami and Maeta 1977, Rehan and Richards in press). Lastly, the similarity in clutch size between species in the group is remarkable (Table 2.9). *Ceratina calcarata* and *C. japonica,* the species nesting at wood margins both had similar clutch sizes of approximately 7.6 and 6.5, both smaller than the 11.5 and 10.4 clutch sizes of the open field nesting *C. calcarata* and *C. dupla* (Sakagami and Maeta 1977).

It appears that these two species pairs share many life history characteristics in common. It would be very interesting to repeat the nest choice experiment with the substrates and microclimate situations typical of *C. flavipes* and *C. japonica* in Japan, to see if these two species would reveal the same results as *C. dupla* and *C. calcarata.* This would lend further insight into whether niche partitioning based on nesting resources may be a common method of reducing interspecific competition in the genus *Ceratina.*

CONCLUSIONS

Ceratina dupla and *C. calcarata* both have the same microhabitat and substrate preferences, sunny sites and raspberry and teasel twigs. The preference for microhabitat is stronger than that for nest substrate. This may be because there are more fitness consequences associated with nesting in shade than there are for nesting in teasel. This is especially true for *C. calcarata,* where clutch size and live brood are greater, and parasitism is lower in sunny nests. In nature, *C. dupla* is found most often in the preferred microhabitat (sun), while *C. calcarata* is found most often in the preferred substrates (raspberry and sumac). Microhabitat has more consequences for *C. calcarata*

and yet *C. dupla* is the species that nests most commonly in the sun, demonstrating that *C. dupla* may be outcompeting *C. calcarata* for sunny nesting sites.

CHAPTER 3: Nest parasitoids of the bee genus *Ceratina* (Hymenoptera: Apidae) in the Niagara Region

J.L. Vickruck, J.T. Huber and M.H. Richards
Submitted to the Journal of the Entomological Society of Ontario, October 2009

INTRODUCTION

Parasite-host relationships have been studied for numerous species in a laboratory setting (Traynor and Mayhew 2005, Harri et al. 2008, Jervis et al. 2008). These studies are vital to help understand the dynamics of host-parasite interactions, however, they often only involve the most common one or two parasitoids associated with the host under study. In a natural setting, hosts may be attacked by a number of parasitoid species at varying frequencies, each using different parasitism and developmental strategies at different times. By describing the life history, development and preferences of numerous parasite species attacking one host, a more complete understanding of these interactions is gained.

Bees of the genus *Ceratina* (often referred to as dwarf carpenter bees) are cosmopolitan, with the subgenus *Zadontomerus* being found exclusively in the Western Hemisphere (Michener 2007). The life history of *Ceratina* offers an excellent opportunity to study the development and interactions of parasites with their hosts. All offspring from eggs laid by a single female can be collected together in a nest, thus allowing for observation of how the parasites interact with an individual host, as well as how nest substrate, position in the nest, and interactions with other parasites and the foundress bee occur.

The Niagara Region, Ontario, Canada, is home to three species of C. (*Zadontomerus*): *C. dupla, C.* near *dupla* and *C. calcarata* and very rare species, *C. strenua.* Their nests are commonly collected from staghorn sumac (*Rhus typhina*), wild raspberry (*Rubus strigosus*) and teasel (*Dipsacus fullonum*) (J. Vickruck, unp. data). Both sumac and wild raspberry are native to the region whereas teasel is an obsolete crop plant introduced from Europe, whose flower heads (when the seeds are mature) were once used to raise the knap on wool (Rector et al. 2006). Sumac and raspberry are both perennial plants found at wood margins, differing from teasel which is a biennial weed found in open, generally abandoned agricultural fields. The objectives of this study were to identify and describe the development of parasites of *Ceratina* in the Niagara Region as well as quantify their host and substrate preferences.

METHODS

Host nest collections

All parasites were reared from a total of 107 nests of *Ceratina calcarata, C. dupla* and *C.* near *dupla* collected from 14 April to 30 September 2008. Supplementary nest collections also took place in June 2009 to aid with final parasite identifications. All collections took place at the Brock University campus (43.1197°N, 79.2492°W), the Glenridge Quarry Naturalization Site (43.1223°N, 79.2375°W) and an abandoned old field site on Glendale Ave. (43.1479°N, 79.1811°W). Nests were collected from sumac, raspberry, and teasel and brought back to the laboratory in early morning to ensure that all occupants were present inside. After being chilled, twigs were carefully split open

longitudinally to identify nest contents. Bee species, plant nest substrate, position of any parasitized cells in the nest, and developmental stages of bees and parasites were recorded on the day of collection. Dissected nests were then inserted in transparent PVC tubing slightly larger than the diameter of the nest (ranging from ½-1 inch depending on twig diameter) for protection and to allow for easy visual observation of nest contents. This also allowed for behavioural observations of host-parasite interactions in the laboratory.

Ceratina species were identified using the key of Rehan and Richards (2008). Parasite identifications were made by Dr. John T. Huber and Dr. Gary Gibson at the Canadian National Collection of Insects, Arachnids and Nematodes (CNC), as well as Jess Vickruck. Voucher specimens of *Baryscapus spp.* 1 and 2, *Eupelmus vesicularis, Coelopencyrtus sp., Axima zabriskiei* and *Eurytoma sp.,* were deposited in the CNC. *Baryscapus* sp. 1, *Coelopencyrtus* sp., *Eupelmus vesicularis* and *Eurytoma* sp. are labelled as CNC Ident. lot # 2008-341, and *Baryscapus* sp. and *Axima zabriskiei* as 2009-188.

Ceratina life history and development

Ceratina in the Niagara region are solitary and univoltine, producing one brood per year and overwintering as newly emerged, unmated adults (J. Vickruck, unp. data). Emergence and mating typically take place in mid-April, and new nests are founded in May. Nests are not reused from year to year and can only be initiated in twigs with exposed pith. After digging a linear tunnel females begin to forage, forming pollen and nectar provisions into rounded masses upon which a single egg is laid (Grothaus 1962,

Kislow 1976, Johnson 1988). Each provision mass and egg is separated from its neighbours by a cell septum formed by the foundress. Once finished provisioning, females sit and guard the nest entrance until the eclosion of their offspring. The newly eclosed adults can either overwinter in their natal nest or disperse to found new hibernacula for the winter (Grothaus 1962, Kislow 1976).

Ceratina immatures were classified into one of the 18 developmental stages originally described by Daly (1966b) for *Ceratina dallatoreana*. The first eight stages rank the larva in relation to the size of the pollen ball, after which the immature passes through a pre-pupal stage followed by metamorphosis. The eyes of the pupa then pass from white through to black (five stages), followed by darkening of the body (four stages). In the final stage the black bodied pupa emerges as an adult with milky wings.

Parasite development and classification

Hosts were observed on a daily basis to detect parasitoid presence. Position in the nest, stage parasitized, and parasitoid species were recorded as soon as they became apparent. Developmental milestones such as defecation, pupation, pigmentation of the exoskeleton and emergence dates were recorded for parasites. Once parasitoids had pupated they were transferred to their own individual 0.2 mL microcentrifuge tubes prior to eclosion. Upon emergence parasitoids were placed in 70% ethanol for later identification.

Parasites were classified as idiobionts or koinobionts, endoparasitoids or ectoparasitoids, and gregarious or solitary. Idiobionts prevent the larva from developing further after initial parasitisation, whereas koinobionts do not kill the host until it has

reached a certain point in the host's development, often the larval or pupal stage. Ectoparasitoids develop outside the host (although they are often attached to it), while endoparasitoids consume the host internally. In solitary species the parasitoid to host ratio is 1:1, whereas in gregarious parasites multiple individuals develop in one host.

RESULTS

Host parasitism

Eight species of arthropod parasitoids representing two classes, two orders, and seven families were reared from a total of 107 *C. dupla* and *C. calcarata* nests containing 840 brood cells. Characteristics of these eight parasitoid species are compared in Table 3.1. Of the 107 nests collected, 64 were teasel, 36 raspberry, and 7 sumac. Twenty-nine percent (249/850) of all brood cells were parasitized, and 30% (32/107) of nests contained at least one parasitoid. *Ceratina* near *dupla* had the highest parasitism rates, followed by *C. calcarata* with *C. dupla* having the lowest parasitism based on the proportion of cells parasitized (Table 3.1). Parasitism for each *Ceratina* species also varied by substrate, with nests in raspberry having significantly higher parasitism rates than those in teasel (Table 3.1). Sumac nests were not included due to small sample size. *Ceratina dupla* nesting in teasel was the least parasitized with 16% of available cells affected (Table 3.1, Fig. 3.1). Only seven sumac nests were found, all *C. calcarata,* in which 48% of immatures had been parasitized (Fig. 3.1). *Ceratina* near *dupla* was only parasitized by one host, but at the highest rate of 60% (24/40) of all immatures.

Table 3.1. Total parasitism for *Ceratina dupla, C.* near *dupla* and *C. calcarata* in each substrate. Due to low sample sizes sumac was excluded from statistical analysis.

Species	Substrate	Prevalence (%) (cells available)	Prevalence (%) (nests available)
C. dupla	Teasel	53/386 (14%)	23/40 (57%)
	Raspberry	24/97 (25%)	10/10 (100%)
	TOTAL	**77/483 (16%)**	**33/50 (66%)**
C. near *dupla*	Teasel	24/40 (60%)	5/9 (56%)
	TOTAL	**24/40 (60%)**	**5/9 (56%)**
C. calcarata	Teasel	32/96 (33%)	9/15 (60%)
	Raspberry	79/198 (40%)	25/26 (96%)
	Sumac	16/33 (48%)	4/7 (57%)
	TOTAL	**127/327 (39%)**	**38/48 (79%)**
***Ceratina* species**		**G=74.02, d.f.=2,P<0.0001**	**G=3.21, d.f.=2, P<0.05**
Teasel vs. Raspberry		**G=18.89, d.f=1, P<0.0001**	**G=22.30, d.f.=1,P<0.0001**

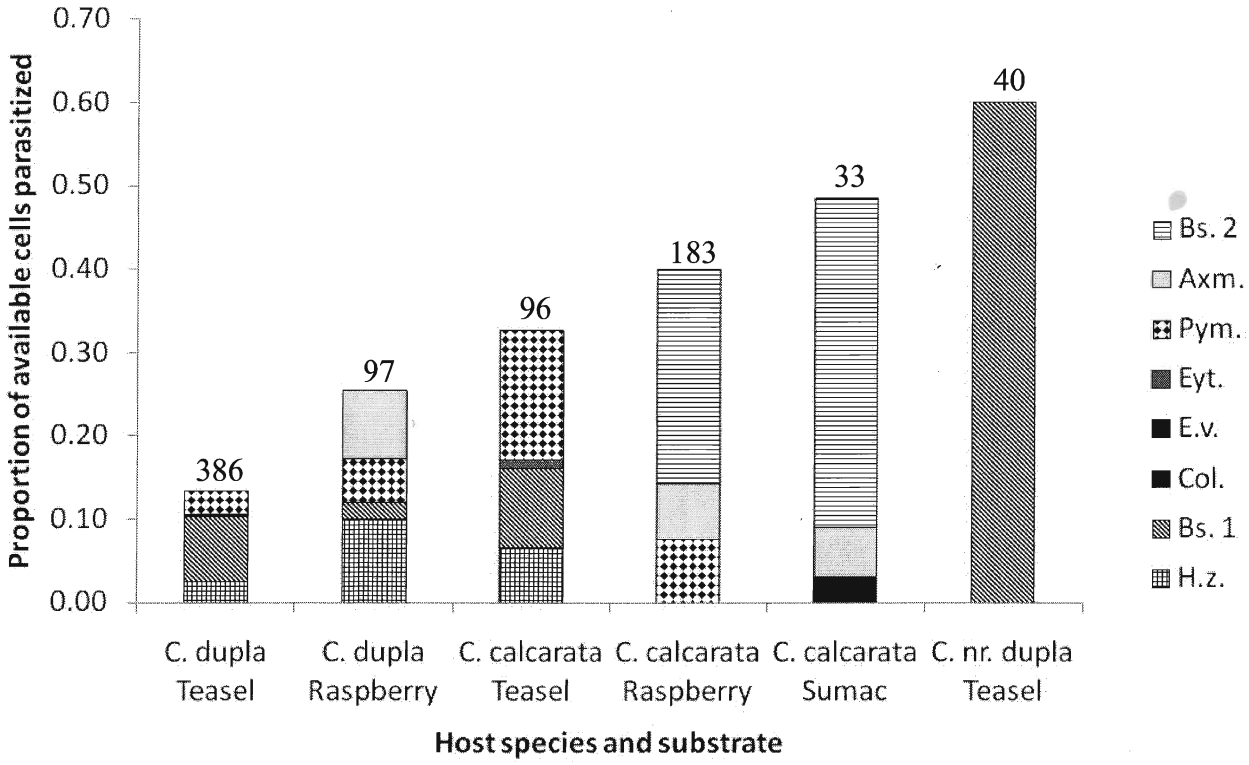


Figure 3.1. The proportion of available cells parasitized for *C. dupla, C.* near *dupla* and *C. calcarata* in each substrate. Values associated with each bar indicate the number of available cells for each species in each substrate. Abbreviations: Bs. 2=*Baryscapus* sp. 2, Axm.=*Axima zabriskiei*, Pym.= *Pyemotes* sp., Eyt.= *Eurytoma* sp., E.v.= *Eupelmus vesicularis,* Col.= *Coelopencyrtus* sp., Bs. 1.=*Baryscapus* sp. 1, H.z.= *Hoplocryptus zoesmairi.*

Parasitoid development

A summary of important parasitoid life history characteristics can be seen in Table 3.2 as well as photographs of most adult parasitoids in Figure 3.2. The frequency and prevalence, i.e., proportion of hosts parasitized, of all eight parasitoids in *Ceratina* nests is presented in Table 3.3 for affected cells and Table 3.4 for infected nests. Detailed observations for each species are given below.

***Hoplocryptus zoesmairi* Dalla Torre (Ichneumonidae)**

This external parasitoid was described taxonomically by Viereck (1904) and biologically by Graenicher (1905) as *Habrocryptus graenicheri,* a parasitoid on *C. dupla.* It was later synonymised with *Hoplocryptus zoesmairi* Dalla Torre (Yu et al. 2005). This is the first time it has been reported as a parasitoid of *C. calcarata.*

There were four occurrences of this parasitoid, two in *C. dupla* nests (one in teasel and one in raspberry), one in a *C. calcarata* nest (raspberry), and one in a *Ceratina* nest that contained no adult female and no surviving offspring. This parasitoid was always laid in the innermost cell of the nest. After the egg hatched, the parasitoid attached to the small *Ceratina* larva, but did not kill it immediately. Rather, the *H. zoesmairi* larva waited until the *Ceratina* larva was at least half as large as its pollen mass, at which point it consumed the immature *Ceratina* and the remainder of its provisions. Once the entire contents of the cell had been consumed the parasitoid broke down the cell septum and consumed the next larva and its pollen mass. This process was repeated, with individual

Table 3.2. Important developmental characteristics of natural enemies of *Ceratina dupla* and *C. calcarata* in the Niagara Region. The species are all Hymenoptera except *Pyemotes* sp. which belongs to Actinedida.

Parasitoid	Type of parasitoid	Host Species	Host nesting substrate	Parasitoids per host	Developmental stage of host	Previous host record
Hoplocryptus zoesmairi (Ichneumonidae)	Idiobiont[a] Ectoparasite	*C. dupla*, *C. calcarata*	Teasel, Raspberry	Predator [b]	Larvae	Reported from *C. dupla* (Viereck 1904, Graenicher 1905), new host record for *C. calcarata*
*Baryscapus sp.*1 (Eulophidae)	Koinobiont Endoparasite	*C. dupla*, *C.* near *dupla*, *C. calcarata*	Teasel, Raspberry	Gregarious (>10)	Prepupae, occasionally white eyed pupae	*B. americanus* reported from *C. calcarata* (Rau 1928, Kislow 1976), new host record for *C. dupla*
Baryscapus sp. 2 (Eulophidae)	Koinobiont Endoparasite	*C. calcarata*	Raspberry, Sumac	Gregarious	Prepupae, white-eyed pupae [d]	See previous host records for *Baryscapus sp.* 1 above
Coelopencyrtus sp. (Encyrtidae)	Koinobiont Endoparasite	*C. calcarata*	Sumac	Gregarious (>20)	Medium larvae	*C. hylaei* reported on *C. calcarata* (Daly 1967)
Eupelmus vesicularis (Eupelmidae)	Koinobiont Ectoparasite	*C. dupla*	Teasel	Solitary	White-eyed pupae	New host record
Eurytoma sp. (Eurytomidae)	Koinobiont Ectoparasite	*C. calcarata* [c]	Teasel	Solitary	Large larva	New host record
Axima zabriskiei (Eurytomidae)	Idiobiont Ectoparasite	*C. dupla*, *C. calcarata*	Raspberry, Sumac	Solitary or Gregarious	Prepupae, white-eyed pupae	*Axima zabriskiei* reported on *C. dupla* and *C. calcarata* (Kislow 1976, Krombien 1960, Rau 1928)
Pyemotes sp. (Pyemotidae)	Idiobiont Ectoparasite	*C. dupla*, *C. calcarata*	Teasel, Raspberry	Gregarious	All larval and pupal stages	New host record for both species

[a] Koinobiont for first larva consumed, idiobiont for those after. [b] Multiple *Ceratina* immatures are consumed in the development of one parasite. [c] May be a hyperparasitoid on *Baryscapus sp* 1.[d] Parasitoids overwinter as full grown larvae.

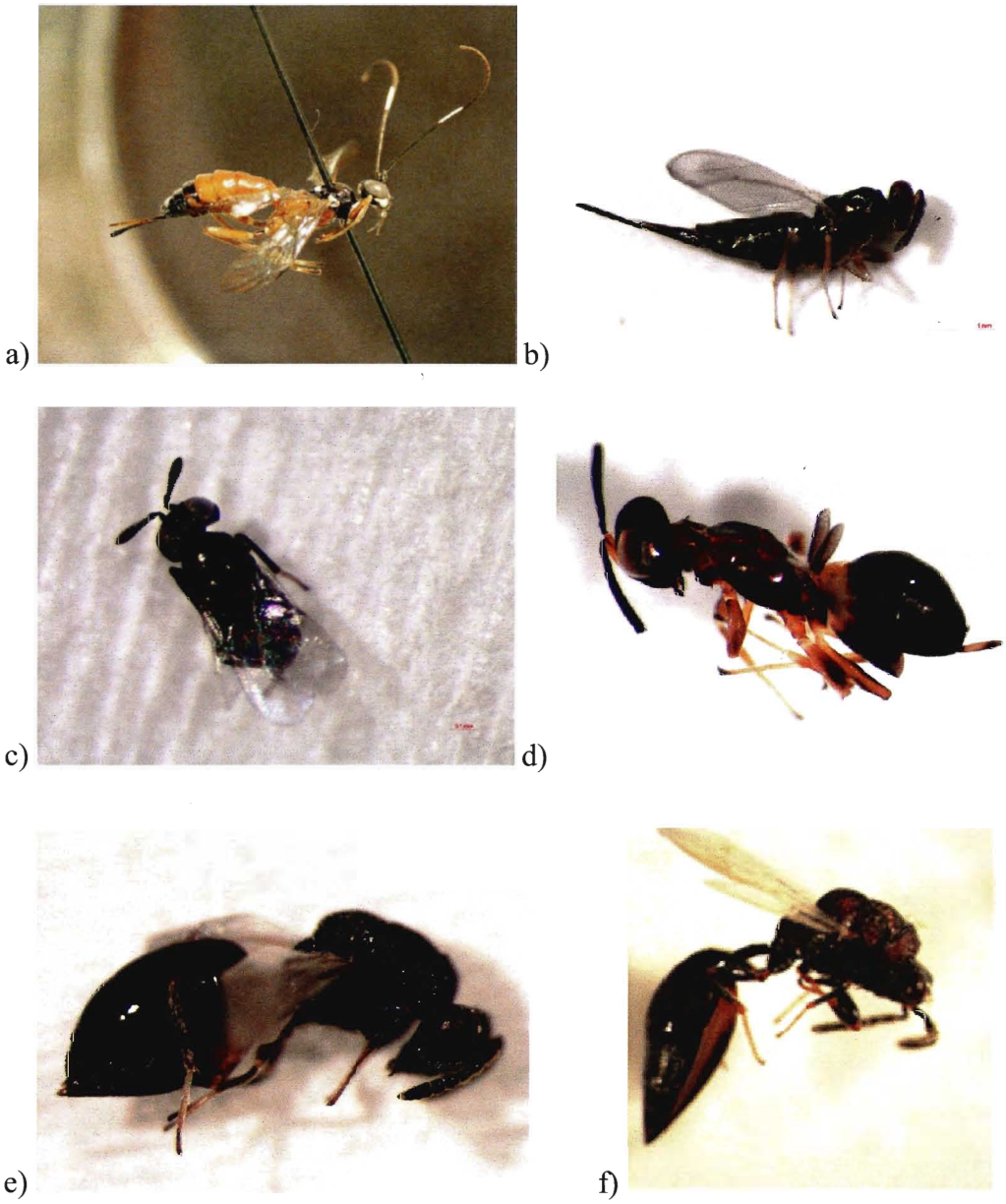


Figure 3.2 Photographs of adult parasitoids reared from *Ceratina* species. a) *Hoplocryptus zoesmairi* b) *Baryscapus sp.* 1 c) *Coelopencyrtus sp.* d) *Eupelmus vesicularis* e) *Eurytoma sp.* f) *Axima zabriskiei.*

Table 3.3. Prevalence of parasitoids on each *Ceratina* host by affected brood cells. Prevalence is the proportion of brood parasitized in each host species in each nesting substrate. *H. zoesmairi* cell data is reported as the number of *Ceratina* that a single parasitoid consumed. Statistics presented wherever possible.

Parasitoid	Host	Substrate	Prevalence (%) cells available	Stats
Hoplocryptus zoesmairi (Ichneumonidae)	*C. dupla*	Teasel	5 larvae	
		Raspberry	2 larvae	
	C. calcarata	Teasel	3 larvae	
	Ceratina sp.	Raspberry	3 larvae	
*Baryscapus sp.*1 (Eulophidae)	*C. dupla*	Teasel	31/386 (13)	***Ceratina* species**
		Raspberry	2/97 (2)	**G=83.50, d.f.=2, P<0.0001**
	C. near *dupla*	Teasel	24/40 (60)	
	C. calcarata	Teasel	9/96 (9)	**Raspberry vs. Teasel**
		Raspberry	0/198 (0)	**G=46.05,d.f=1, P<0.0001**
		TOTAL	**42/777 (5)**	
Baryscapus sp. 2 (Eulophidae)	*C. calcarata*	Raspberry	51/198 (26)	Raspberry vs. Sumac
		Sumac	13/33 (40)	G=2.48, d.f.=1, n.s.
		TOTAL	**64/231 (28)**	
Coelopencyrtus sp. (Encyrtidae)	*C. calcarata*	Sumac	1/33 (3)	
Eupelmus vesicularis (Eupelmidae)	*C. dupla*	Teasel	1/426 (>1)	
Eurytoma sp. (Eurytomidae)	*C. calcarata*	Teasel	1/96 (1)	
Axima zabriskiei (Eurytomidae)	*C. dupla*	Raspberry	8/97 (8)	*C. calcarata* vs. *C. dupla*
		Sumac	0/0 (0)	G=0.17, d.f.=1, n.s.
	C. calcarata	Raspberry	14/198 (7)	Raspberry vs. Sumac
		Sumac	2/33 (6)	*X^2=0.9, d.f.=1, n.s.
		TOTAL	**24/328 (3)**	
Pyemotes sp. (Pyemotidae)	*C. dupla*	Teasel	51/386 (12)	*C. calcarata* vs. *C. dupla*
		Raspberry	5/97 (5)	G=0.36, d.f.=1, n.s.
	C. calcarata	Teasel	15/96 (16)	**Raspberry vs. Teasel**
		Raspberry	15/198 (8)	**G=4.80, d.f.=1, P<0.03**
		TOTAL	**86/777 (11)**	

*Fisher's exact

Table 3.4. Prevalence of parasitoids on each *Ceratina* host by affected nests. Prevalence is the proportion of affected nests in each host species in each nesting substrate. Statistics presented wherever possible.

Parasitoid	Host	Substrate	Prevalence (%) Nests available	Stats
Hoplocryptus zoesmairi (Ichneumonidae)	*C. dupla*	Teasel	1/40 (3)	
		Raspberry	1/10 (10)	
	C. calcarata	Teasel	1/15 (7)	
	Ceratina sp.	Raspberry	1/36 (all rasp. nests)	
Baryscapus sp. 1 (Eulophidae)	*C. dupla*	Teasel	8/40 (27)	***Ceratina* species**
		Raspberry	2/10 (20)	**G=12.79, d.f.=2, P=0.002**
	C. near *dupla*	Teasel	5/9 (56)	
	C. calcarata	Teasel	2/15 (13)	**Raspberry vs. Teasel**
		Raspberry	0/26 (0)	**G=6.03, d.f.=1, P=0.01**
		TOTAL	**12/91 (13)**	
Baryscapus sp. 2 (Eulophidae)	*C. calcarata*	Raspberry	16/26 (62)	Raspberry vs. Sumac
		Sumac	2/7 (14)	*X^2=1.07, d.f.=1, n.s.
		TOTAL	**18/33 (55)**	
Coelopencyrtus sp. (Encyrtidae)	*C. calcarata*	Sumac	1/7 (14)	
Eupelmus vesicularis (Eupelmidae)	*C. dupla*	Teasel	1/49 (2)	
Eurytoma sp. (Eurytomidae)	*C. calcarata*	Teasel	1/15 (1)	
Axima zabriskiei (Eurytomidae)	*C. dupla*	Raspberry	4/10 (40)	*C. calcarata* vs. *C. dupla*
		Sumac	0/0 (0)	G=0.90, d.f.=1, n.s.
	C. calcarata	Raspberry	7/26 (27)	Raspberry vs. Sumac
		Sumac	1/7 (14)	G=1.21, d.f.=1, n.s.
		TOTAL	**12/43 (11)**	
Pyemotes sp. (Pyemotidae)	*C. dupla*	Teasel	13/40 (27)	*C. calcarata* vs. *C. dupla*
		Raspberry	3/10 (30)	G=2.73, d.f.=1, n.s.
	C. calcarata	Teasel	5/15 (33)	**Raspberry vs. Teasel**
		Raspberry	2/26 (8)	**G=4.33, d.f.=1, P=0.04**
		TOTAL	**23/91 (25)**	

* Fisher's Exact

H. zoesmairi parasitoids devouring anywhere from two to five *Ceratina* immatures and pollen masses, then spinning silken cocoons. Each *H. zoesmairi* larva then defecated and pupated inside its cocoon before emerging as an adult. Development from time of hatching to adulthood took 27-48 days, with emergence dates ranging from 28 July to 14 August 2008. This external parasitoid is a koinobiont from the perspective of the juvenile bee in the innermost cell, as it did not kill the host immediately, but would be considered an idiobiont to the other parasitized bees in the nest as it consumed them immediately, regardless of developmental stage.

***Baryscapus sp.* 1 (Eulophidae)**

Baryscapus americanus (Ashmead) was previously known to parasitize C. *calcarata* in Georgia (Kislow 1976) and Missouri (Rau 1928). The species was transferred from the genus *Aprostocetus* by Lasalle (1994). This is the first record of any member of the genus *Baryscapus* parasitizing *C. dupla* and *C.* near *dupla.*

Baryscapus sp. 1 is a gregarious, koinobiont endoparasitoid of *Ceratina* immatures. Their presence was undetectable until they began to consume their hosts (Fig. 3.3a), but the larvae grew to approximately half the length of their *Ceratina* host by the time its contents had been entirely consumed. At this point the parasitoids migrated to the anterior or posterior ends of the larval skin (Fig. 3.3b). Three groups of parasitoids then emerged: either all individuals in the *Ceratina* larval skin pupated and emerged that summer, or all of the individuals remained as prepupa to overwinter together and emerge the following spring, or several individuals occupying a single host would pupate while

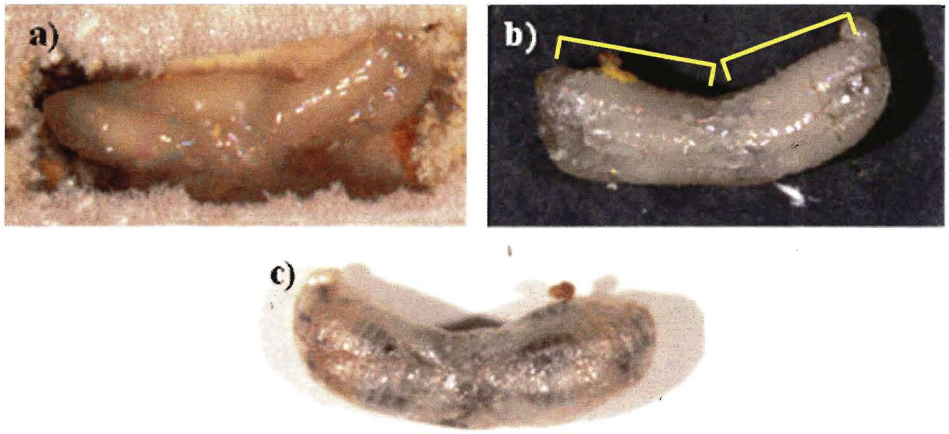


Figure 3.3. Development of *Baryscapus sp.* 1 a) Parasitoid larvae consume the contents of the *Ceratina* immature, leaving the larval skin. b) Full grown larvae move to the anterior and posterior ends of the host (yellow brackets). c) Thereafter, pupation and development continue to eclosion or individuals overwinter as prepupae.

the rest would overwinter. The aforementioned strategies were also observed by Kislow (1976). Of the 65 immature *Ceratina* parasitized, 20 (31%) showed total emergence, 35 (54%) overwintered as a group together, and 10 (15%) showed partial emergence, with some individuals emerging that summer and some overwintering as prepupae. Average development time was 21.6 ± 2.3 days (range 11–37) once *Baryscapus sp.* 1 larvae had begun to consume *Ceratina* immatures. Emergence was highly synchronized for non-diapausing larvae, with all newly eclosed adults emerging from the host within 24 hours.

Baryscapus sp. 1 was the second most common parasitoid species observed, infecting 8% (66/850) of all cells, and 16% (17/107) of all nests. They were most often found parasitizing nests in teasel, with low levels of infection in raspberry, and none in sumac (Tables 3.3, 3.4). On average they infected 39% of available brood in an affected nest, ranging from one immature to the entire nest. This parasitoid predominantly affected the prepupal stage (8/9 *C. calcarata,* 28/31 *C. dupla* and of 24/24 *C.* near *dupla*) and occasionally white eyed pupae. Individuals of *Baryscapus* sp. 1 were often found in nests with other parasitoid species (7/17, 41%), including *Eurytoma* sp., *Axima zabriskiei, Eupelmus vesicularis* and *Pyemotes* sp.

***Baryscapus sp.* 2 (Eulophidae)**

This parasitoid, which mummifies its host, overwintered as prepupae in the larval or pupal skin of *Ceratina calcarata* only. All individuals of this gregarious, koinobiont endoparasitoid that emerged as adults were male. It infected 8% (64/842) of the total

cells available and 16% (17/107) of all nests. It was found most commonly in raspberry (51 of 64 cells), occasionally in sumac (13 of 64 cells), and never in teasel. On average 3.8 ± 0.6 cells per affected nest were parasitized, representing 51% of infected *C. calcarata* nests on average. Other parasitoids were present in 8 of the 17 infected nests (47%); these were always *Pyemotes* or *Axima*. Prepupae were the most commonly affected host stage (43/64), but white-eyed pupae (21/64) were also susceptible to parasitism.

Parasitism went unnoticed until these internal parasitoids began to consume the host. Infection became evident when the larval skin of the *C. calcarata* changed dramatically in colour and consistency. The larval skin of living *Ceratina* is somewhat transparent and the gut is often visible. Parasitism caused the larval skin of the *Ceratina* to become a rusty red-brown colour; it also became much more brittle with the consistency of paper maché. The parasitoids overwintered as full grown larvae in the host, and the tough pupal casing of the larval or pupal skin may provide protection to the diapausing larvae (Legrand et al. 2004). Only males of this species emerged as adults from *Ceratina* immatures, in contrast with *Baryscapus* sp. 1 where both sexes emerged.

***Coelopencyrtus* sp. (Encyrtidae)**

A single *Ceratina calcarata* larva in a sumac nest was affected by this gregarious, endoparasitic koinobiont. The only other observation of *C. calcarata* being attacked by *Coelopencyrtus* is by R.W. Matthews (reported by Daly *et al.* 1967), who reported *Coelopencyrtus hylaei* parasitism on six consecutive cells in a nest collected in

Connecticut. *Coelopencyrtus* have also been reported to parasitize members of the twig-nesting, bee genus *Hylaeus* (Burks 1958).

The *C. calcarata* nest was collected on 7 July 2008 and parasitism became evident on 10 July 2008 when more than 20 *Coelopencyrtus* larvae could be seen consuming the bee larva, which was in the second innermost cell in a nest with six other immatures. Once the entire contents of the *Ceratina* larva had been consumed, development of the parasitoids continued inside the transparent larval skin. Eyes of the parasitoids began to darken on 4 August with their exoskeletons gaining pigmentation by 7 August. Synchronized emergence took place on 13 August, when all of the new *Coelopencyrtus* adults emerged, except for one individual that had died during development.

***Eupelmus vesicularis* Retzius (Eupelmidae)**

One *Eupelmus vesicularis* specimen was reared from a *Ceratina dupla* nest in teasel. While this is the first host record of *E. vesicularis* parasitizing *C. dupla,* members of the genus *Eupelmus* are well known for parasitizing a large number of different hosts (Burks 1979, Gibson 1990). *Eupelmus vesicularis* has a Holarctic distribution, but may have been introduced to North America from Europe in straw (Burks 1979). Its first record in North America was from Pennsylvania in 1915 (Burks 1979).

Usually a primary parasitoid, *E. vesicularis* has been occasionally reported as a secondary parasitoid (Burks 1979). The wasp collected here had actually parasitized a white-eyed bee pupa that had also been parasitized by *Baryscapus* sp 1. The *E.*

vesicularis egg had already been laid when the nest was collected on 15 July 2008. The parasitoid hatched and began feeding externally on the bee larva on 20 July 2008. A day later it became apparent that the bee larva had also been parasitized internally by *Baryscapus* sp. 1. *Eupelmus vesicularis* consumed the bee larva, followed by the *Baryscapus* sp. 1 parasitoids, and pupated on 1 August. Body sclerotization was quite rapid, beginning 4 August and finishing 2 days later. The adult *E. vesicularis* emerged on 8 August 2008, 19 days after first hatching.

***Eurytoma* sp. (Eurytomidae)**

This is the first record of a member of the genus *Eurytoma* parasitizing *C. calcarata. Eurytoma apiculae* Bugbee and *E. nodularis* Boheman were reported as parasitoids on other species of *Ceratina* in California (Bugbee 1966, Daly 1966a), and an unknown *Eurytoma* species has been observed as a parasitoid of *C. australensis* in Queensland, Australia (S. Rehan, pers. comm.).

An external parasitoid of *C. calcarata,* only one *Eurytoma* individual was collected which was parasitizing a larva that had almost finished eating its pollen ball in a nest constructed in teasel. The *Eurytoma* egg was laid in the innermost brood cell and by 16 July, 2008, had begun to feed on the host *Ceratina* larva. Over the course of the next week the parasitoid finished consuming the host, after which it defecated and then pupated. The eyes of the *Eurytoma* began to darken on 27 July and the integument was fully pigmented by 1 August. The teneral adult emerged on 3 August, 2008.

***Axima zabriskiei* Howard (Eurytomidae)**

Axima zabriskiei has been reported as a parasitoid of both *C. dupla* and *C. calcarata* (Rau 1928, Krombein 1960, Kislow 1976). An ectoparasitic idiobiont, 1–7 *Axima* individuals could be seen consuming a single *Ceratina* immature, always a pre-pupa or white eyed pupa, most often attached between the head and thorax and/or near the wing buds of white eyed pupae (Fig 3.4b). The parasitoids consumed the hosts' contents rapidly (usually in 24-48 hours), leaving the skin intact (Fig 3.4c). It was at this point that most lab-reared parasitoids died, but two did pupate in the laboratory in 2008 (Fig. 3.4d). None of these chalcid parasitoids were successfully reared to adulthood in the lab in 2008 but one was reared to adulthood during 2009 collections.

Axima zabriskiei parasitoids infected 3% (23/842) of all available cells and 11% (12/107) of available nests. Twenty-one of the infected cells were found in raspberry (11 nests) and two cells were in sumac (one nest), for an average of 1.9 ± 0.3 cells per infected nest, with a maximum of four infected *Ceratina* immatures but never representing more than 50% of the total brood in a nest. *A. zabriskiei* was found with other parasitoids in 7/12 (58%) affected nests, most often in conjunction with *Baryscapus sp.* 2.

***Pyemotes* sp. (Actinedida: Pyemotidae)**

Pyemotes sp. were the most common parasitoids found on *Ceratina* immatures, infecting 10% (86/842) of all available brood cells and 21% (23/107) of all available nests. This is the first record of *Pyemotes* mites infecting *C. dupla* and *C. calcarata,* although they have been reported on *C. dallatoreana* in California (Daly 1966a). They

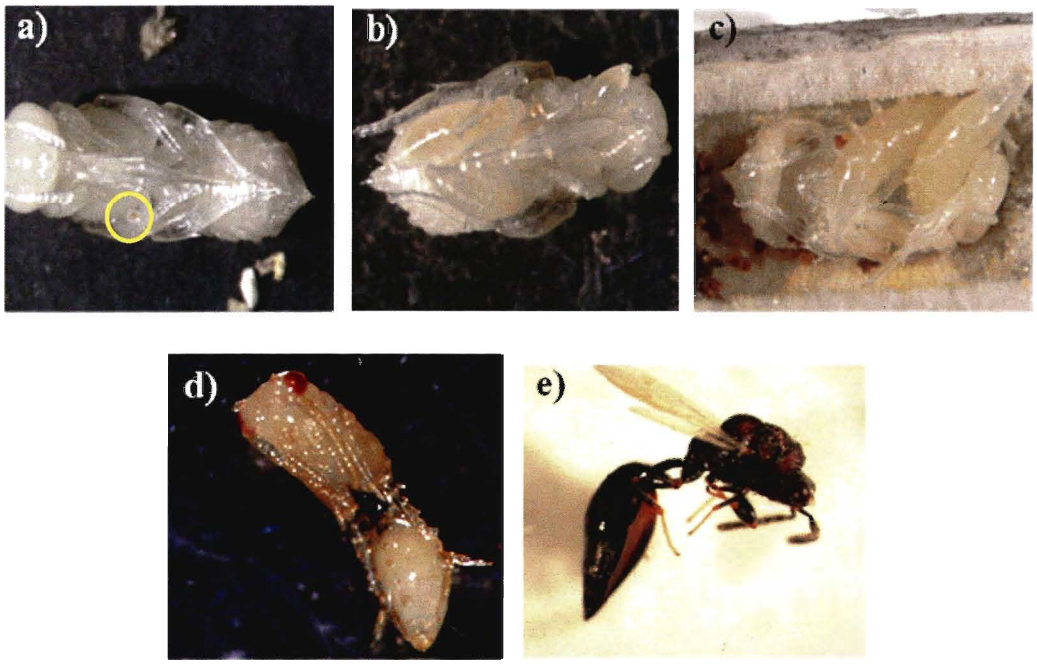


Figure 3.4. *Axima zabriskiei* wasp development. a) Newly hatched parasitoids (inside yellow circle) pierce the soft exoskeleton of the pupa and rapidly ingest the contents, usually within 24-48 hours. Often multiple parasitoids will attack a single *Ceratina* immature (b and c). Once finished feeding larvae pupates (d) before emerging as an adult (e).

were more common in teasel nests (67 of 86 infected brood) than in raspberry (19 of 86 infected brood), and were not found in sumac (Table 3). On average *Pyemotes* affected 3.7 ± 0.7 immatures per nest, representing 28% of the total brood in affected nests.

This external parasitoid was found to infect all immature stages, from small larvae to fully pigmented pupae. Multiple individuals often infected a single larva or pupa, but a single mite was effective in killing the host. *Pyemotes* seemed to monopolize parasitism in a nest, being found with other parasitoids only 22% of the time (5/23 nests). *Pyemotes* mites also killed two *A. zabriskiei* larvae. Other members of the genus *Pyemotes* have been known to destroy nests of the bee *Melipona colimana* Ayala and the stem-nesting wasp, *Psenulus interstitalis* Cameron (Matthews 2000, Macias-Macias and Otero-Colina 2004).

DISCUSSION

Oviposition methods of parasitoids

Because *Ceratina* mothers are nest loyal, guarding their nests once they have finished provisioning and laying eggs, opportunities for invaders to enter their nests are limited. Moreover, *Ceratina* mothers also open brood cells to inspect immatures, (Kislow 1976, Sakagami and Maeta 1977), and this could provide opportunities to identify parasitized brood cells. There are several strategies that parasitoids can employ to overcome these defences and oviposit in bee nests. First, a female parasitoid can inject her eggs through the nest substrate via her ovipositor, a strategy exhibited by many

parasitoids that lay eggs through tree bark (Spradbery 1970, Nenon 1995, Quicke et al. 2005). Second, the parasitoid can enter through the nest entrance and lay her eggs on the cells inside while the foundress is foraging. Third, the female can acquire parasitoid eggs or individuals while foraging outside the nest, and then transfer them to her brood cells by phoresy, either when the nest is being constructed or when she re-opens the cells for inspection (Schwarz and Huck 1997).

All three of these strategies are probably used by parasitoids of *C. dupla* and *C. calcarata*. Individuals of *Baryscapus* and *Axima* spp. have been found ovipositing through stems into *Ceratina* nests (Kislow 1976). Although this behaviour was not observed during our study, parasitized nests were collected that had a linear set of punctures down the outside of the twig. This method of parasitism would be very effective to circumvent both *Ceratina dupla* and *C. calcarata* mothers guarding the nest entrance. It is also an extremely effective method of parasitisation as eggs can be laid in many cells sequentially without having to break down the cell partitions that isolate eggs inside the nest.

Although no adult parasitoids were collected in nests in our study, direct entry of the parasitoid into the nest to lay eggs seems likely for some of the species we reared. For example, *Hoplocryptus zoesmairi* was always found in the innermost cell of the nest, implying that the parasitoid must enter the nest to determine whether this is the first brood cell. Such behaviour may help to ensure that there are multiple *Ceratina* immatures available to consume in the nest, as this parasitoid always ate several hosts before completing development.

Phoresy is the strategy most likely used by *Pyemotes.* These parasitoids are small and flightless so encountering a nest by locomotion is unlikely. Some species of *Pyemotes* have two female morphs, one phoretic and one physogastric (Cross and Moser 1975, Moser and Cross 1975). Only the physogastric morph is parasitic on the immature host. As *Pyemotes* only appear to infect immature stages of *Ceratina* a female phoretic morph that enters the nest via the bee foundress might be able to crawl through the cell septa due to her small size.

The mode of parasitism for *Coelopencyrtus* sp. is less obvious. Its eggs were most likely laid in the larva itself, which indicated that this parasitoid entered the nest to oviposit. The ovipositor of *Coelopencyrtus* is also too short to penetrate the twig (Daly et al. 1967) and there were no external punctures visible on the twigs themselves associated with the presence of this parasitoid.

Nest substrate and parasitism rates

Both *C. dupla* and *C. calcarata* were least parasitized in teasel, with higher parasitisation in raspberry and, although few nests were collected, the highest parasitisation was *C. calcarata* in sumac (Table 3.2; Fig. 3.1). Many parasitoids were more prevalent in one substrate than in another. *Baryscapus sp.* 1 for example, was collected significantly more often from teasel nests, with only two cells parasitized in raspberry (Table 3.4). While not statistically significant *Axima zabriskiei* was collected most often from nests laid in raspberry (Table 3.4). *Pyemotes* mites did not parasitize one species more than another, but were significantly more common in teasel nests then they were in raspberry (Tables 3 and 4). While *Baryscapus* sp. 2 was found parasitizing 64

individuals in 33 nests, it was only ever a parasite of *C. calcarata* in raspberry and sumac, never in teasel. The parasitoid preferences seen for specific host substrates may be due to a number of factors such as the structure or biology of the host plant itself.

The following discussion pertains mainly to raspberry and teasel, because so few sumac nests were collected. One possible reason for higher parasitism rates in raspberry than in teasel may be the structure of the plant species used for *Ceratina* nests. Teasel nests can only contain one nest per plant, in the straight stalk that grows perpendicular to the ground. Shrubs like raspberry (and sumac) have multiple branches in each plant and thus multiple possible nest substrates. These shrubs also tend to grow in aggregations, with multiple plants in very close proximity to one another. This can lead to higher nest densities in raspberry than in teasel. As *Ceratina dupla* and *C. calcarata* females guard only their own nests, the high density of nests in shrubs may lead to increased rates of parasitism, as an individual parasitoid may be able to efficiently locate and infect several nests in close proximity. When comparing parasitism rates for a number of non-social hymenopteran species that nest solitarily and in aggregations, Rosenheim (1990) found that aggregated nests had higher parasitism rates in most cases.

Higher parasitism rates in raspberry may also relate to the habitat and biology of the plants in which *Ceratina* nest. Teasel is an invasive plant found in large open fields, almost always in full sunlight, while raspberry and sumac are both native plants located in shaded wood margins. In other words, *Ceratina* are nesting in different microclimates, in substrates with different biology, and with different possible chemical signatures. Numerous experiments have shown that many parasitoids are attracted to chemical cues of the flora where their host species are commonly found (Vet 1983, Drost et al. 1986,

Elzen et al. 1986, Godfray 1994). If parasitoids use the microclimate and/or chemical cues emitted by the native substrates, then this might explain why the nests in native shrubs had higher parasitisation. Members from the genus *Eupelmus* parasitize a very wide range of host species (Gibson 1990). Gibson (1990) hypothesized that *Eupelmus* searched for hosts in specific microclimates, with the microclimate being of more importance than the host species. Searching for hosts by their preferred substrate may also be more effective in temperate regions due to the relatively short and synchronized phenology of foraging insects and nest substrates (Wcislo 1987).

General conclusions and further directions

The first chapter of this thesis revealed that the *Ceratina* community in the Niagara Region is composed of four species: *Ceratina dupla, C. calcarata* and a previously unknown species morphologically similar to *C. dupla,* referred to here as *C.* near *dupla,* as well as the extremely rare *C. strenua.* Both *C. dupla* and *C. calcarata* are quite common each making up nearly 49% of the local population, while *C.* near *dupla* is relatively rare, comprising only 2%. While all three species appear superficially very similar, closer examination reveals that there are subtle differences and that they occupy slightly different ecological niches. The data obtained by outlining the basic nesting biology of these species will provide the backbone for all future studies on *C. dupla* and *C.* near *dupla.*

The second chapter closely investigated the components of nest site selection and competition between the two common *Ceratina* in Niagara (*C. dupla* and *C. calcarata*). Through a combination of nest collections in 2008 and a nest choice experiment in 2009, it was determined that although *C. dupla* and *C. calcarata* have the same site and substrate preference if given all options, in nature *C. dupla* nests predominantly in the preferred site, while *C. calcarata* nests in the preferred substrate (predominately in raspberry and teasel twigs). This demonstrates that there is competition for nesting sites among the *Ceratina* in the Niagara region, and that *C. dupla* and *C. calcarata* are partitioning resources to help reduce this interspecific competition. This is aided by the fact that both *C. dupla* and *C. calcarata* immatures are able to upregulate development

when nesting in the shade (i.e. when nesting in raspberry or sumac), an ability that allows for them to occupy a wider range of nest sites.

The third chapter of this thesis shifted the focus onto the interactions of *Ceratina* with their parasitoids. *Ceratina dupla* and *C. calcarata* in the Niagara Region are parasitized by no less than eight different species of parasitoid. Seven of these are aculeate Hymenoptera, with one species of physogastric mite. These parasites used many different oviposition strategies and had different developmental life histories, from external solitary predators that needed to consume multiple *Ceratina* individuals to complete development, to internal gregarious parasitoids that could develop upwards of 20 individuals inside one *Ceratina* host. Not only did this study provide many new parasitoid host records and biological data on parasitoid species, but stressed the importance that parasitism in this group happens from a wide range of parasitoid strategies, an important factor to consider when looking to model experimental effects of a single parasitoid species on a host.

From here there are many avenues to investigate. Sparked by Chapters One and Two, it would be very interesting to know more about the biogeography of the *Zadontomerus* of eastern North America moving along a latitudinal gradient from north to south. For instance, how does community composition change when the length of the nesting season increases, especially for the bivoltine *C.* near *dupla?* Preliminary data also suggests that there may be other cryptic species of *C. dupla* in eastern North America and that *C.* near *dupla* is a relatively new species (Rehan and Sheffield in prep). By understanding the nesting biology, site and substrate preferences of a newly emerging species group, we may be able to add to our knowledge of the mechanism of speciation.

From a methodological standpoint, we also now know that we can entice *Ceratina* to nest in a somewhat artificial setting. All three species of *Ceratina* nested in the twigs provided at the experimental sites in 2009. By knowing where specific nests are located, one would be able to find the same nests on repeated days. This is a powerful tool, as individual nests could be watched intensively for foraging data, as well as interactions with conspecifics and parasites.

References

Banschbach, V. S., N. Levit, and J. M. Herbers. 1997. Nest temperatures and thermal preferences of a forest ant species: Is seasonal polydomy a thermoregulatory mechanism? Insectes Sociaux **44**:109-122.

Barthell, J. F., J. M. Hranitz, R. W. Thorp, L. M. Overall, and J. L. Griffith. 2004. Heat stress responses of an invasive leafcutting bee species nesting in the central valley of California: A case of risky maternal behavior? Pages 521-521. Soc Integrative Comparative Biology.

Bereczki, J., K. Pecsenye, L. Peregovits, and Z. Varga. 2005. Pattern of genetic differentiation in the *Maculinea alcon* species group (Lepidoptera, Lycaenidae) in Central Europe. Journal of Zoological Systematics and Evolutionary Research **43**:157-165.

Blanckenhorn, W. U. 1991. Life-history differences in adjacent water strider populations: phenotypic plasticity or heritable responses to stream temperature? Evolution **46**:1520-1525.

Bosch, J. 1994. Comparison of nesting materials for the orchard pollinator *Osmia cornuta* (Hymenoptera: Megachilidae). Entomologia Generalis **19**:285-289.

Bugbee, R. E. 1966. A new species of the genus *Eurytoma* Illiger parasitic on bees of the genus *Ceratina* Latreille. The Pan-Pacific Entomologist **42**:210-211.

Burks, B. D. 1958. A recharacterization of the genus *Coelopencyrtus*, with descriptions of two new species (Hymenoptera: Encyrtidae). Journal of the Washington Academy of Sciences **48**:22-26.

Burks, B. D. 1979. Family Eupelmidae. Pages 878-889 *in* K. V. Krombein, P. D. Hurd, D. R. Smith, and B. D. Burks, editors. Catalogue of Hymenoptera in America north of Mexico. Smithsonian Institution Press, Washington D.C.

Chandler, L. 1975. Eusociality in *Ceratina calcarata* Robertson (Hymenoptera: Anthophoridae). Pages 283-284 *in* Proceedings of the Indiana Academy of Science.

Colla, S. R. and L. Packer. 2008. Evidence for decline in eastern North American bumblebees (Hymenoptera : Apidae), with special focus on Bombus affinis Cresson. Biodiversity and Conservation **17**:1379-1391.

Comstock, A. B. 1911. Handbook of nature study. 24 edition. Comstock Publishing Associates, Ithica.

Conover, D. O. and E. T. Schultz. 1995. Phenotypic similarity and the evolutionary significance of countergradient variation. Trends in Ecology and Evolution **10**:248-252.

Cross, E. A. and J. C. Moser. 1975. New, dimorphic species of *Pyemotes* and a key to previously described forms (Acarina, Tarsonemoidea). Annals of the Entomological Society of America **68**:723-732.

da Cruz, J. D., E. Giannotti, G. M. M. Santos, C. C. Bichara, and A. A. da Rocha. 2006. Nest site selection and flying capacity of neotropical wasp *Angiopolybia pallens* (Hymenoptera : Vespidae) in the Atlantic Rain Forest, Bahia state, Brazil. Sociobiology **47**:739-749.

Daly, H. V. 1966a. Biological studies on *Ceratina dallatoreana*, an alien bee in California which reproduces by parthenogenesis (Hymenoptera: Apoidea). Annals of the Entomological Society of America **59**:1138-1154.

Daly, H. V. 1966b. Biological studies on *Ceratina dallatorreana*, and alian bee in California which reproduces by parthenogenesis. Annals of the Entomological Society of America **59**:1138-1154.

Daly, H. V. 1973. Bees of the Genus *Ceratina* in America North of Mexico. University of California Press, Berkeley, California.

Daly, H. V., G. I. Stage, and T. Brown. 1967. Natural enemies of bees of the genus *Ceratina* (Hymenoptera: Apoidea). Annals Entomological Society of America **60**:1273-1282.

Dejean, A., J. Orivel, B. Corbara, I. Olmsted, and J. P. Lachaud. 2001. Nest site selection by two polistine wasps: The influence of *Acacia-Pseudomyrmex* associations against predation by army ants (Hymenoptera). Sociobiology **37**:135-146.

Drost, Y. C., W. J. Lewis, P. O. Zanen, and M. A. Keller. 1986. Beneficial arthropod behaviour mediated by airborne semiochemicals. 1. Flight behaviour and influence of preflight handling of *Microplitis croceipes* (Cressson). Journal of Chemical Ecology **12**:1247-1262.

Elton, C. 1927. Animal Ecology. Sidgwick and Jackson, London.

Elzen, G. W., H. J. Williams, and S. B. Vinson. 1986. Wind-tunnel flight responses by Hymenpterous parasitoid compoletis-sonorensis to cotton cultivars and lines. Entomologia Experimentalis et Applicata **42**:285-289.

Friesen, V. L., A. L. Smith, E. Gomez-Diaz, M. Bolton, R. W. Furness, J. Gonzalez-Solis, and L. R. Monteiro. 2007. Sympatric speciation by allochrony in a seabird. Proceedings of the National Academy of Sciences of the United States of America **104**:18589-18594.

Gause, G. F. 1932. Ecology of populations. The Quarterly Review of Biology **7**:27-46.

Gause, G. F. 1934. The struggle for existence. Hafner publishing company, New York.

Gibson, G. A. P. 1990. Revsison of the genus *Macroneura* Walker in America north of Mexico (Hymenoptera: Eupelmidae). The Canadian Entomologist **122**:837-873.

Godfray, H. J. C. 1994. Parasitoids. Princeton University Press, Princeton, New Jersey.

Goulson, D. and B. Darvill. 2004. Niche overlap and diet breadth in bumblebees; are rare species more specialized in their choice of flowers? Apidologie **35**:55-63.

Goulson, D., G. C. Lye, and B. Darvill. 2008. Diet breadth, coexistence and rarity in bumblebees. Biodiversity and Conservation **17**:3269-3288.

Graenicher, S. 1905. On the habits of two Ichneumonid parasites of the bee *Ceratina dupla* Say. Entomological News **16**:43-49.

Graham, L. and K. N. Jones. 1996. Resource partitioning and per-flower foraging efficiency in two bumble bee species. The American Midland Naturalist **136**:401-406.

Grant, P. R. 1986. Ecology and Evolution of Darwin's Finches. Princeton University Press, New Jersey.

Grimmell, J. 1917. The niche-relationships of the California thrasher. The Auk **34**:427-433.

Grixti, J. C., L. T. Wong, S. A. Cameron, and C. Favret. 2009. Decline of bumble bees (Bombus) in the North American Midwest. Biological Conservation **142**:75-84.

Grothaus, H. G. 1962. The biology of the species of *Ceratina* (Hymenoptera, Xylocopidae) in Indiana. Purdue University.

Harri, S. A., J. Krauss, and C. B. Muller. 2008. Natural enemies act faster than endophytic fungi in population control of cereal aphids. Journal of Animal Ecology **77**:605-611.

Heatwole, H. and D. M. Davis. 1965. Ecology of three sympatric species of parasitic insects of the genus *Megarhyssa* (Hymenoptera, Ichneumonidae). Ecology **46**:140-150.

Hranitz, J. M. and J. F. Barthell. 2003. Heat shock protein 70 during development of the leafcutting bee, Megachile rotundata (Hymenoptera : Megachilidae). Southwestern Entomologist **28**:97-103.

Hranitz, J. M., J. F. Barthell, R. W. Thorp, L. M. Overall, and J. L. Griffith. 2009. Nest Site Selection Influences Mortality and Stress Responses in Developmental Stages of Megachile apicalis Spinola (Hymenoptera: Megachilidae). Environmental Entomology **38**:484-492.

Hutchinson, E. G. 1957. Population studies: Animal ecology and demography. Cold Spring Harbour Symposia on Quantitative Biology **22**:415-427.

Inouye, D. W. 1978. Resource partitioining in bumblebees: experimental studies on foraging behaviour. Ecology **59**:672-678.

Jervis, M. A., J. Ellers, and J. A. Harvey. 2008. Resource acquisition, allocation, and utilization in parasitoid reproductive strategies. Annual Review of Entomology **53**:361-385.

Johnson, M. D. 1988. The relationship of provision weight to adult weight and sex ratio in the solitary bee, *Ceratina calcarata*. Ecological Entomology **13**:165-170.

Johnson, M. D. 1990. Female size and fecundity in the small carpenter bee, *Ceratina calcarata* (Robertson) (Hymenoptera: Anthophoridae). Journal of the Kansas Entomological Society **63**:414-419.

Johnson, R. A. 1986. Intraspecific resource partitioning in the bunble bees *Bombus ternarius* and *B. pensylvanicus*. Ecology **67**:133-138.

Kim, J. 1999. Influence of resource level on maternal investment in a leaf-cutter bee (Hymenpotera: Megachilidae). Behavioral Ecology **10**:552-556.

Kislow, C. J. 1976. The comparative biology of two species of small carpenter bee, *Ceratina strenua* F. Smith and *C. calcarata* Robertson (Hymenoptera: Xylocopinae). University of Georgia, Athens, Georgia.

Krombein, K. V. 1960. Biological notes on some Hymenoptera that nest in sumach pith. Entomological News **71**:29-36.

Kurihara, M., Y. Maeta, K. Chiba, and S. F. Sakagami. 1981. The relation between ovarian conditions and life cycle in two small carpenter bees, *Ceratina flavipes*

and *C. japonica* (Hymenoptera, Anthophoridae). Journal of the Faculty of Agriculture, Iwate University **15**:131-149.

Lasalle, J. 1994. North American genera of Tetrastichinae (Hymenoptera, Eulophidae). Journal of Natural History **28**:109-236.

LeBuhn, G., T. Griswold, R. Minckley, S. Droege, T. Roulston, J. Cane, F. Parker, S. Buchmann, V. Tepedino, N. Williams, C. Kreman, and O. Messenger. 2003. A standardized method for monitoring Bee Populations- The Bee Inventory (BI) Plot.

Legrand, M. A., C. Salin, A. Langer, and T. Hance. 2004. Are mummy characteristics reliable indicators of diapause and cold tolerance in the parasitoid wasp *Aphidius rhopalosiphi* (Braconidae, Aphidiinae)? Cryoletters **25**:161-166.

Macias-Macias, J. O. and G. Otero-Colina. 2004. Infestation of *Pyemotes tritici* (Acari: Pyemotidae) on *Melipona colimana* (Hymenoptera: Apidae: Meliponinae): A case study. . Agrociencia **38**:525-528.

Marcil, J., D. P. Swain, and J. A. Hutchings. 2006. Countergradient variation in body shape between two populations of Atlantic cod (*Gadus morhua*). Proceedings of the Royal Society B-Biological Sciences **273**:217-223.

Matsumoto, R. and K. Konishi. 2007. Life histories of two Ichnuemonid parasitoids of *Cyclosa octotuberculata* (Araneae): *Reclinervellus tuberculatus* (Uchida) and its new sympatric congener (Hymenoptera:Ichneumonidae:Pimpinae) Entomological Science **10**:267-278.

Matthews, R. W. 2000. Nesting biology of the stem-nesting wasp *Psenulus interstitialis* Cameron (Hymenoptera: Crabronidae: Pemphredoninae) on Magnetic Osland, Queensland. Austalian Journal of Entomology **39**:25-28.

McEvoy, P. B. 1986. Niche partitioning in spittlebugs (Homoptera: Cercopidae) sharing shelters on host plants. Ecology **67**:465-478.

McIntosh, M. 1996. Nest-substrate preferences of the twig-nesters *Ceratina acantha*, *Ceratina nanula* (Apidae) and *Pemphredon lethiefer* (Sphecidae). Journal of the Kansas Entomological Society **69**:216-231.

Michener, C. D. 2007. The bees of the world. Johns Hopkins University Press, Baltimore, Maryland.

Moser, J. C. and E. A. Cross. 1975. Phoretomorph- A new phoretic phase unique to Pyemotidae (Acarina, Tarsonemoidea). Annals of the Entomological Society of America **68**:820-822.

Murray, T. E., M. Kuhlmann, and S. G. Potts. 2009. Conservation ecology of bees: populations, species and communities. Apidologie **40**:211-236.

Nenon, J. P. 1995. Bioecology of *Megarhyssa atrata* [Hymenoptera : Ichneumonidae], an ectoparasitoid of *Tremex columba* [Hymenoptera: Siricidae] in Quebec. Phytoprotection **76**:115-122.

Peterson, J. H. and B. D. Roitberg. 2006a. Impact of resource levels on sex ratio and resource allocation in the solitary bee, *Megachile rotundata*. Environmental Entomology **35**:1404-1410.

Peterson, J. H. and B. D. Roitberg. 2006b. Impacts on flight distance on sex ratio and resource allocation to offspring in the leafcutter bee, *Megachile rotunda*. Behav Ecol Sociobiol **59**:589-596.

Plateaux-Quenu, C. 1992. Comparative biological data in the closely related eusocial species, *Evylaeus calceatus* (Scop.) and *Evylaeus albipes* (F.) (Hym, Halictinae). Insectes Sociaux **39**:351-364.

Polidori, C., P. Mendiola, J. D. Asis, J. Tormos, J. Selfa, and F. Andrietti. 2008. Female-female attraction influences nest establishment in the digger wasp *Stizus continuus* (Hymenoptera : Crabronidae). Animal Behaviour **75**:1651-1661.

Potts, S. G., B. Vulliamy, A. Dafni, G. Ne'eman, and P. Willmer. 2003. Linking bees and flowers: how do floral communities structure pollinator communities? Ecology **84**:2628-2642.

Potts, S. G., B. Vulliamy, S. Roberts, C. O'Toole, A. Dafni, G. Ne'eman, and P. Willmer. 2005. Role of nesting resources in organising diverse bee communities in a Mediterranean landscape Ecological Entomology **30**:78-85.

Potts, S. G. and P. Willmer. 1997. Abiotic and biotic factors influencing nest-site selection by *Halictus rubicundus*, a ground-nesting halictine bee. Ecological Entomology **22**:319-328.

Quicke, D. L. J., N. M. Laurenne, and M. Barclay. 2005. Host, host location and aggressive behaviour in a tropical wood-borer parasitoid genus *Monilobracon* Quicke (Hymenoptera: Braconidae), parasitoids of Lymexylidae (Coleoptera) in Kibale Forest National Park, west Uganda. African Entomology **13**:213-220.

Quinn, T. P., M. J. Unwin, and M. T. Kinnison. 2000. Evolution of temporal isolation in the wild: Genetic divergence in timing of migration and breeding by introduced chinook salmon populations. Evolution **54**:1372-1385.

Rau, P. 1928. The nesting habits of the little carpenter bee, *Ceratina calcarata*. Annals of the Entomological Society of America **21**:380-396.

Rector, B. G., V. Harizanova, R. Sforza, T. Widmer, and R. N. Wiedenmann. 2006. Prospects for biological control of teasels, *Dipsacus* spp., a new target in the United States. Biological Control **36**:1-14.

Rehan, S. M. and M. H. Richards. 2008. Morphological and DNA sequence delineation of two problematic species of *Ceratina* (Hymenoptera: Apidae) from Eastern Canada. Journal of the Entomological Society of Ontario **139**:59-67.

Rehan, S. M. and M. H. Richards. 2010. Nesting biology and subsociality in the small carpenter bee, *Ceratina calcarata* (Hymenoptera: Apidae). The Canadian Entomologist.

Rehan, S. M. and M. H. Richards. in press. Nesting biology and subsociality in the small carpenter bee, *Ceratina calcarata* (Hymenoptera: Apidae). Canadian Entomologist.

Rice, W. R. 1987. Speciation via habitat specialization: the evolution of reproductive isolation as a correlated character. Evolutionary Ecology **1**:301-314.

Rosenheim, J. A. 1990. Density-dependent parasitism and the evolution of aggregated nesting in the solitary Hymenoptera. Annals of the Entomological Society of America **83**:277-286.

Rutgers-Kelly, A. 2003. The bees of Niagara: a test of the intermediate disturbance hypothesis. Brock University, St. Catharines, Ontario.

Sakagami, S. F. and Y. Maeta. 1977. Some presumably presocial habits of Japanese *Ceratina* bees, with notes on various social types in Hymenoptera. Insectes Sociaux **24**:319-343.

Schultz, E. T., K. E. Reynolds, and D. O. Conover. 1996. Countergradient variation in growth among newly hatched *Fundulus hererclitus:* geographic differences revealed by common-environment experiments. Functional Ecology **10**:366-374.

Schwarz, H. H. and K. Huck. 1997. Phoretic mites use flowers to transfer between foraging bumblebees. Insectes Sociaux **44**:303-310.

Sheffield, C. S., P. D. N. Hebert, P. G. Kevan, and L. Packer. 2009. DNA barcoding a regional bee (Hymenoptera: Apoidea) fauna and its potential for ecological studies. Molecular Ecology Resources **1**:196-207.

Shiokawa, M. 1963a. Comparative study of two closely allied sympatric *Ceratina* bees, *C. flavipes* and *C. japonica*. II. Nest structure. Kontyu **34**:44-51.

Shiokawa, M. 1963b. Redescriptions of *Ceratina flavipes* Smith and *C. japonica* Cockerell (Hymenoptera, Apidae). Kontyu **31**:276-280.

Sielezniew, M. and A. M. Stankiewicz. 2007. Differences in the development of the closely related myrmecophilous butterflies *Maculinea alcon* and *M. rebeli* (Lepidoptera: Lycaenidae). European Journal of Entomology **104**:433-444.

Skelly, D. K. 2004. Microgradient countergradient variation in the wood frog *Rana sylvatica*. Evolution **58**:160-165.

Smith, E. F. 2004. Nest sites of the paper wasp *Mischocyttarus collarellus* (Hymenoptera : Vespidae : Polistinae) in a lowland tropical rain forest. Journal of the Kansas Entomological Society **77**:457-469.

Spradbery, J. P. 1970. Host finding by *Rhyssa persuasoria* (L), an ichneumonid parasite of sircid woodwasps. Animal Behaviour **18**:103-&.

Tepedino, V. J. and F. D. Parker. 1983. Nest size, mortality and Sex-Ratio in *Osmia marginata* Michener (Hymenoptera, Megachilidae). Southwestern Entomologist **8**:154-167.

Tepedino, V. J. and F. D. Parker. 1984. Nest selection, mortality and sex-ratio in *Hoplitis fulgida* (Cresson) (Hymenoptera, Megachilidae). Journal of the Kansas Entomological Society **57**:181-189.

Tepedino, V. J. and P. F. Torchio. 1989. Influence of nest hole selection on sex-ratio and progeny size in *Osmia lignaria propinqua* (Hymenoptera, Megachilidae). Annals of the Entomological Society of America **82**:355-360.

Toler, T. R., E. W. Evans, and V. J. Tepedino. 2005. Pan-trapping for bees (Hymenoptera : Apiformes) in Utah's West Desert: the importance of color diversity. Pan-Pacific Entomologist **81**:103-113.

Traynor, R. E. and P. J. Mayhew. 2005. Host range in solitary versus gregarious parasidoids: a laboratory experiment. Entomologia Experimentalis et Applicata **117**:41-49.

Tuell, J. K., A. K. Feildler, D. Landis, and R. Isaacs. 2008. Visitation by wild and managed bees (Hymenoptera:Apoidea) to Eastern U.S. native plants for use in conservation programs. Community and Ecosystem Ecology **37**:707-718.

Turlure, C., H. Van Dyck, N. Schtickzelle, and M. Baguette. 2009. Resource-based habitat definition, niche overlap and conservation of two sympatric glacial relict butterflies. Oikos **118**:950-960.

Vet, L. E. M. 1983. Host-habitat location through olfactory cues by *Leptopilina clavipes* (Hartig)(Hym, Eucoilidae), a parasitoid of fungivorous *Drosophila* - the influence of conditioning. Netherlands Journal of Zoology **33**:225-248.

Viereck, H. L. 1904. A new cryptine from the nest of *Ceratina dupla*. Entomological News **15**:333-335.

Wcislo, W. T. 1987. The roles of seasonality, host synchrony, and behaviour in the evolution and distributions of nest parasites in Hymenoptera (Insecta), with special reference to bees (Apoidea). Biological Reviews of the Cambridge Philosophical Society **62**:515-543.

Wuellner, C. T. 1999. Nest site preference and success in a gregarious, ground-nesting bee Dieunomia triangulifera. Ecological Entomology **24**:471-479.

Yasumatsu, K. and Y. Hirashima. 1969. Synopsis of the small carpenter bee genus *Ceratina* of Japan (Hymenoptera, Anthophoridae). Kontyu **37**:61-70.

Yu, D. S., C. van Achterberg, and K. Horstmann. 2005. World Ichneumonoidea 2004-Taxapad.